SODIUM AND POTASSIUM IN AUSTRALIAN FOODS

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Although restricted sodium diets are often prescribed, values for the sodium and potassium content of Australian foodstuffs do not appear to be available, with the exception of the results of a survey of some Queensland foods for mineral content undertaken by Lee *et al.* (1938). These figures appeared in an appendix to a report of the Commonwealth Advisory Council on Nutrition (1938). The sodium and potassium were estimated colorimetrically by McCance and Shipp's method (1933), by precipitating the sodium as sodium uranyl zinc acetate, and the potassium as potassium cobaltinitrite. In analysing some 500 American foods for sodium and potassium, Bills *et al.* (1949a) used a flame photometer and obtained values lower than those obtained by McCance and Widdowson (1947), who used the above colorimetric method. In the opinion of a Committee of the Food and Nutrition Board, National Research Council, U.S.A., the lower values obtained by using the newer method are more nearly correct.

Materials and Methods.

Accordingly, approximately 150 Australian foods were estimated for sodium and potassium with an "EEL" flame photometer.

The instrument error, based on 20 readings when the standard solution contained 10 p.p.m., was found to be:

Concentration p.p.m.	1	5	9
Na—Mean error, %	3·8	1·41	0·73
K—Mean error, %	3·7	2·27	0·38

Interference by potassium on sodium readings and vice versa was evaluated.

All the materials were ashed, following Bills' (1949b) procedure, except for the cordials, fruit juices and milk (human and cow) samples which were read directly or diluted with distilled water before they were read in the flame photometer. The analyses were done in duplicate and generally two samples of each foodstuff were taken. When the two samples were widely different in sodium and/or potassium content, then one or more further samples were estimated (Table 1).

[1]This work was instigated by the Nutrition Committee of the National Health and Medical Research Council.

TABLE 1.

Sodium and potassium content of edible portions of some Australian foods.

	No. of Samples	Moisture %	Sodium		Potassium	
			Mean	Range	Mean	Range
			mg./100 gm.		mg./100 gm.	
Cereals and Cereal Products						
Biscuits						
Arrowroot	2	3	190	160-220	110	100-110
Pat-a-Cake	2	4	190	180-200	100	80-120
Ry-Vita	2	4	520	420-610	375	340-410
Sao	2	3	780	770-790	140	120-160
Scotch Finger	2	4	380	340-410	130	125-135
Thin Captain	2	4	610	590-630	160	130-190
Vita-Weat	3	3	500	475-525	290	270-310
Wheatmeal	2	3	470	460-470	220	210-230
Bran	1	15	5	—	1300	—
Bread						
Brown	2	36	580	490-670	115	110-120
White	2	37	580	540-620	110	110-115
White, low sodium	1	34	400	—	150	—
Breakfast foods						
All Bran	2	8	1440	1420-1460	950	860-1040
All Wheat biscuits	2	8	620	550-680	320	310-330
Cornflakes	2	3	1050	1050-1050	130	130-130
Rice Bubbles	2	2·5	1020	980-1060	105	100-110
San Bran	2	8	600	550-650	1180	890-1470
Weet-Bix	2	8	280	260-300	400	360-440
Farex	2	4	190	170-210	120	50-190
Flour						
Plain	2	12	2·2	1·3-3·1	125	120-130
Self-raising, phosphate	2	12	740	680-800	140	120-160
Oatmeal	3	9	11	1-27	300	280-320
Pollard	1	14	4	—	600	—
Rice						
Flour	1	13	1·4	—	120	—
Polished and ground	2	13	1·5	1·3-1·7	115	110-120
Scones	2	22	1290	1240-1340	270	270-270
Wheat and Wheatmeal	1	10	2·1	—	310	—
Wheat Germ						
Bemax	2	5	3·8	3·3-4·3	900	870-930
Betta B	2	5	15	10-20	1140	1140-1140
Vitos	2	6	6	4-8	1320	940-1700
Weat-Harts	2	6	8	7-9	1050	970-1130
Fats and Oils						
Cod liver oil	2	0	2	1-3	10	6-13
Dripping, beef	2	1	1·4	0·6-2·2	5	3-6
Lard	2	1	0·5	0·4-0·5	1·3	0·8-1·7
Fish						
Bream	1	78	90	—	380	—
Bream, sea	1	78	30	—	230	—
Cod, smoked	2	75	1430	1280-1570	200	195-205
Flounder, frozen	2	77	20	20-20	60	55-65
Oysters, quick frozen	2	76	380	370-380	210	200-220
Sardines in oil, canned						
Optimist Brand	1	57	1480	—	920	—
Seakist Brand	1	57	250	—	390	—
Other Norwegian Brand	1	57	740	—	460	—
Scallops, frozen	2	76	190	160-210	380	340-410

TABLE 1 (*continued*).

	No. of Samples	Moisture %	Sodium		Potassium	
			Mean	Range	Mean	Range
			mg./100 gm.		mg./100 gm.	
Fruits						
Apples, less skin and core	2	86	1·4	1·4-1·4	100	100-100
Apricots, dried	2	21	20	20-20	2090	1840-2340
Bananas	2	71	2·3	1·5-3	430	350-510
Lemons, juice only	3	91	12	4-20	145	100-190
Oranges, juice only	2	87	2·5	2·3-2·6	130	120-140
Prunes	2	28	7	6-7	930	920-940
Raisins	2	15	75	70-80	940	850-1030
Rhubarb	3	94	20	5-40	170	150-190
Sultanas	2	15	30	26-31	1000	950-1050
Tomatoes	2	94	3	2-3	270	250-290
Meats						
Bacon						
44% Fat, raw	1	37	2360	—	160	—
53% Fat, raw	1	37	1680	—	120	—
Beef						
Corned, cooked	2	63	1740	1530-1950	150	100-200
Round Steak, raw	2	76	70	65-75	280	250-310
Round Steak, grilled						
Meat, no drippings	2	64	70	65-70	360	320-400
Meat, plus drippings	2	50	115	115-115	550	535-565
Drippings only (3% of weight of meat)	1	6	1030	—	4670	—
Ham	2	44	1960	1700-2220	155	150-160
Lamb						
Chump Chops, raw	2	67	70	65-70	320	250-390
Chump Chops, grilled						
Meat, no drippings	2	56	70	55-90	315	300-330
Meat, plus drippings	2	56	85	70-100	480	430-530
Drippings only (4% of weight of meat)	1	6	340	—	1470	—
Neck Chops, raw	1	68	80	—	280	—
Neck Chops, stewed ½ hr. in ½ pint water	1	51	40	—	220	—
Neck Chops, stewed ½ hr. in 1 pint water	1	55	30	—	120	—
Mutton						
Neck Chops, raw	1	70	60	—	300	—
Neck Chops, stewed ½ hr. in ½ pint water	1	53	60	—	210	—
Neck Chops, stewed ½ hr. in 1 pint water	1	58	50	—	180	—
Milk and Dairy Products						
Butter	2	16	650	635-670	67	56-78
Low sodium	1	17	12	—	25	—
Casinal	2	7	90	80-100	30	20-40
Cheese						
Cheddar, block, mild	3	37	520	310-650	70	70-70
Cheddar, block, tasty	2	37	585	560-610	50	40-60
Cream	2	50	130	80-170	370	280-460
Kraft	2	37	1810	1730-1890	60	55-65
Maxam	2	37	1210	990-1420	50	40-60
Velveeta	3	37	1650	1280-2070	210	180-230

TABLE 1 (*continued*).

	No. of Samples	Moisture %	Sodium		Potassium	
			Mean	Range	Mean	Range
			mg./100 gm.		mg./100 gm.	
Eggs						
Whites only	2	88	165	140-190	110	100-125
Whole	3	74	130	90-200	130	110-150
Yolks only	2	49	30	25-35	70	60-80
Ice Cream	2	64	110	80-130	170	170-170
Milk						
Cows'						
Condensed, sweetened	2	26	130	120-140	360	300-425
Condensed, unsweetened	2	72	110	100-115	300	290-300
Dried (Glaxo No. 2)	2	5	350	330-360	940	850-1020
Dried (Lactogen)	2	4	430	360-500	930	850-1000
Dried, Skim	2	3·5	670	520-810	1570	1500-1630
Dried (Sunshine)	2	4	360	330-390	1060	1000-1120
Fresh	2	88	50	50-50	140	110-160
Human						
Colostrum, 24 hrs. post-partum	3	87	150	70-230	50	40-60
14 days post-partum	3	86	24	17-30	60	55-60
25-35 days post-partum	3	88	23	15-30	55	48-65
40 days and more post-partum	3	88	12	10-16	49	47-51
Whey						
Skim (made from dried milk)	3	96	100	90-120	85	80-90
Whole (made from fresh milk)	2	92	135	120-150	145	145-145
Miscellaneous						
Arobon	1	5	40	—	860	—
Bonox	2	35	750	690-810	1020	890-1150
Bovril	2	38	500	440-550	1910	1460-2350
Chocolate						
Light	2	1	90	80-100	350	310-390
Dark (Cadbury's)	2	0	100	95-105	440	390-480
Dark (Nestle's)	2	0	11	10-12	400	280-520
Drinking (Cadbury's)	2	2	430	400-460	410	360-460
Coca-Cola	2	91	3	2·4-3·3	1·7	1·2-2·1
Cocoa						
Cadbury's	2	3	440	420-460	410	360-460
Bournville	3	3	1070	710-1270	1010	490-1290
Coffee						
Nescafe	2	4	19	18-19	3950	3460-4440
Ricory	2	4	190	180-200	1580	1560-1600
Roasted beans	2	0	4	3-4	1830	1520-2140
Golden Syrup	2	14	100	90-110	980	960-1000
Honey	2	23	6	5-6	40	25-55
Jam, plum	2	29	13	9-17	65	50-80
Jelly, raspberry	2	2	17	15-19	7	6-8
Junket						
Tablets	2	0	35,250	33,500-36,995	180	70-290
Made up (1 tablet to ½ pint milk)	2	83	115	100-130	125	120-130
Lemonade	2	89	1	1-1	0·9	0·8-1·0
Marmite	2	31	5200	4700-5700	1250	1120-1380

TABLE 1 (*continued*).

	No. of Samples	Moisture %	Sodium		Potassium	
			Mean	Range	Mean	Range
			mg./100 gm.		mg./100 gm.	
Robsoup	1	11	620	—	1050	—
Soda Water	2	100	0·6	0·5-0·7	0·25	0·2-0·3
Soups						
Heinz Bone and Vegetable Broth	4	83	200	130-280	180	120-280
Heinz Chicken Broth	2	88	340	280-400	65	60-70
Nestle's Chicken Broth	2	88	200	160-230	130	120-140
Spices, mixed	2	3	120	100-145	790	750-830
Sugar						
Brown	4	3	30	10-60	300	110-600
White	2	0	1·6	0·7-2·5	5	5-5
Tea	2	0	13	12-13	1730	1680-1780
Treacle	3	21	100	80-120	840	540-1260
Vegemite	2	32	4090	3540-4640	1550	1520-1570
Vinegar	2	100	2	2-2	30	25-35
Water (from N.S.W. water supplies)	5	100	1	0·6-1·4	0·06	0·04-0·08
Nuts						
Coconut, desiccated	2	0	13	12·5-13	420	260-570
Peanut butter	2	4	320	260-385	650	630-660
Peanuts, roasted	2	4	5	2-7	540	530-550
Vegetables						
Beans						
Green, fresh	2	92	16	12-20	1770	1560-1980
Green, frozen	2	92	15	11-18	800	790-810
Haricot	2	8	4	2-6	1280	1180-1380
Lima	2	13	4	2-6	1470	1380-1560
Beetroot	4	87	50	20-120	280	270-290
Cabbage	4	92	20	2-50	240	130-300
Capsicums (green peppers)	3	92	2·4	1·6-4·0	220	210-240
Carrots	4	90	110	40-200	270	190-340
Cauliflowers						
Fresh	3	92	10	5-20	300	230-390
Frozen	2	92	13	11-14	200	190-210
Celery	2	94	170	160-180	410	340-480
Lettuce	3	95	30	13-55	110	110-110
Peas						
Fresh	3	79	4	1-8	370	240-600
Frozen	3	79	380	250-510	160	150-190
Potatoes	2	78	1·5	1·5-1·5	350	350-350
Pumpkin	2	88	2	2-2·6	290	230-340
Silver beet (Spinach)	3	91	650	450-790	480	380-650

The sodium and potassium content of a sample of wheat and its various products collected from a local flour mill was estimated (Table 2). The wheat was f.a.q. bulk wheat from the West Wyalong and Grong Grong districts, N.S.W., and had a protein content of 9·5 p.c. The flour contained 8·5 p.c. protein (N×5·7).

TABLE 2.

Item	Sodium mg./100 gm.	Potassium mg./100 gm.
Wheat	2·5	340
1st Break		
Flour	2·3	130
Coarse Semolina	2·0	180
Fine Semolina	1·8	140
Break Meal	2·4	150
Fine Middlings	2·0	144
Coarse Middlings	1·8	130
2nd Break		
After grinding	2·4	410
3rd Break		
Before grinding	4·3	820
After grinding	4·4	950
4th Break		
After grinding	5·0	1250
Bran	6·2	1500
Pollard	4·85	700
Flour, after bleaching	1·3	140

DISCUSSION.

As expected, the widest ranges in sodium content were found in fruit and vegetables. When our sodium figures are compared with those of Lee (1938), who used the colorimetric method, beans, peas, potatoes, pumpkin and tomatoes have values ten to thirty times lower.

When analysed by the flame photometer, Australian apples, apricots, bananas, beans, carrots, celery, lemon and orange juices, lettuce, peas, pumpkin, raisins, rhubarb and spinach appear to have a somewhat higher sodium content, and beetroot, cabbage and cauliflower a lower sodium content than the corresponding American fruit and vegetables (Bills *et al.*, 1949a). But, with the exception of silver beet (spinach), which is considerably higher (650 mg. p.c. sodium as compared with 82 mg. p.c.), the American figures do not lie very far out of the range obtained by us.

It is of interest that of Nestle's two soluble coffee preparations, Ricory has about ten times more sodium and half as much potassium as Nescafe.

In some of our processed foods such as ham, corned beef, bacon, peanut butter and frozen peas, there is obviously more added salt than in the corresponding American products.

The water samples were all collected from dams supplying Sydney and the metropolitan area, with the exception of a sample from the South Coast supply which contained about half the amount of sodium and potassium that was present in the other four samples. Figures for the sodium and potassium content of some public water supplies in the other States were forwarded to

the Institute and found to be so low that it was not considered worth while to carry out any more analyses. However, it is quite possible that well and bore waters may contain more sodium.

The results of the sample of wheat and its products are in fairly close agreement with those obtained by Howe and Sullivan (1936), although they obtained greater values for the potassium content of wheat. Schrenk and King (1948) analysed spectrographically three varieties of Kansas-grown wheat over a period of three years and found the sodium content to be 5–10 times higher than most other published figures. It is of interest that wheat of high mineral content had the higher protein values. Thus the lower sodium content of the analysed sample of Australian wheat may be a reflection of the low protein content of that particular season's wheat.

It is emphasized that the relatively small number of samples taken in the present survey are intended to serve only as a guide to the range of the amounts of sodium and potassium present in the various foods analysed.

SUMMARY.

Values for the sodium and potassium content of approximately 150 Australian foods, as determined by the flame photometer, are presented.

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THE ABSORPTION OF FATTY CHYLE AND ARTIFICIAL FAT EMULSIONS FROM THE PERITONEAL CAVITY

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The physiological significance of lymphatic drainage from serous cavities has been discussed in a recent review by Courtice and Simmonds (1954). While an extensive literature exists on both the qualitative and quantitative aspects of the absorption of particulate and colloidal material from the peritoneal cavity, little information is available concerning the rate at which fat is removed. Results following the injection of fixed oil (Schwartze, 1921) and emulsified radioactive fat (Rutenberg, Seligman and Fine, 1949), suggested that peritoneal fat absorption was a slow and incomplete process. In view of the known rapid rates of removal of physiological substances such as proteins and red cells, the peritoneal absorption of fat present in thoracic duct lymph as chylomicrons and as a prepared olive oil emulsion has been investigated.

Methods.

Absorption rates were studied in rats and guinea-pigs, using the intraperitoneal injection and recovery technique described previously (Morris, 1953). Lymph for injection was obtained from fat-fed rats and cats by cannulation of the thoracic duct. In rats, the technique used was that described by Bollman, Cain and Grindlay (1948), while thoracic duct lymph was collected from cats by direct cannulation of the duct at its venous entry, using glass cannulae.

An artificial fat emulsion was prepared by recirculating homogenization at high pressure, using a mixture containing 6 p.c. glucose, approx. 10 p.c. olive oil and 1 p.c. Tween 80 (polyoxyethylene sorbitan monoleate) as an emulsifying agent. The average particle size of the preparation was less than 1 μ and the emulsion remained stable after autoclaving. The total fatty acid content of the emulsion used was measured as 367·9 meq./l., whilst the thoracic duct lymph samples injected varied in fat content between 5·1 meq./l. and 207·4 meq./l.

Plasma for injection was obtained from rats and cats by venepuncture and stored overnight in the refrigerator. Blood samples for analysis were taken by cardiac puncture and the plasma removed by centrifugation. Powdered heparin (Evans) was used throughout as an anticoagulant. The blue dye, T-1824, was added to lymph and plasma as a label for the

[1]This work was carried out with the aid of a grant from the National Health and Medical Research Council, Canberra.

proteins. Intraperitoneal injections were made at the rate of 20 ml./kg. for plasma, lymph and prepared emulsion, and 4 mg./kg. for T-1824.

Chemical analyses. Total esterified fatty acid was estimated by the colorimetric method of Sterne and Shapiro (1953), the optical densities being recorded at 520 mμ on a Beckman Quartz model DU spectrophotometer. T-1824 concentrations were measured after extraction from plasma and lymph, using the method of Allen (1951), modified for fatty samples (Korner, Morris and Courtice, 1954). Total protein concentrations were estimated on injected and recovered fluids by micro-kjeldahl digestion and direct nesslerization, the optical densities being measured with a "Spekker" photoelectric absorptiometer.

RESULTS.

The rates at which homologous and heterologous fatty chyle and the artificial fat emulsion were absorbed from the peritoneal cavity of the rat are shown in Fig. 1. All three types of emulsion were removed rapidly and completely, but there were significant differences in their rates of absorption. Two samples of homologous lymph with total fatty acid contents of 12·6 and 147·0 meq./l. were absorbed in about 3 hours, whereas samples of cat lymph containing 75·1 and 207·4 meq./l. required 5–6 hours for complete removal. The presence of fat

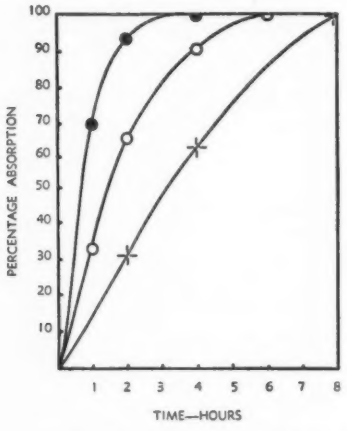

Fig. 1. The absorption of homologous and heterologous fatty chyle and prepared fat emulsion in the rat following intraperitoneal injection, 20 ml./kg. body weight.

● Homologous chyle.
○ Heterologous chyle.
\+ Prepared emulsion.

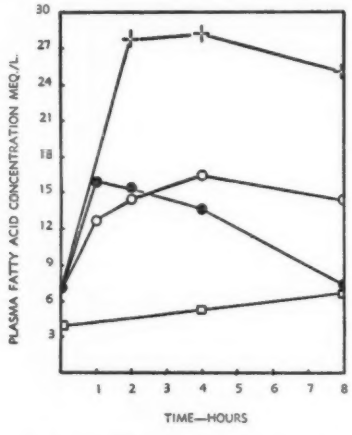

Fig. 2. The plasma fatty acid concentration in the rat and guinea-pig, during the absorption of fat from the peritoneal cavity.

Rat—
● Homologous chyle, 141 meq./l. total fatty acid.
○ Heterologous chyle, 207 meq./l. total fatty acid.
\+ Prepared emulsion, 367·9 meq./l. total fatty acid.

Guinea-pig—
□ Prepared emulsion, 367·9 meq./l. total fatty acid.

in the lymph in these concentrations did not affect the absorption time, as high and low fat samples were removed at approximately the same rate. The prepared emulsion was absorbed at the slowest rate, requiring about 8 hours for complete removal. These differences in absorption rate in the rat were statistically significant ($P=<0{\cdot}001$) when tested by an analysis of variance. The rate of absorption of the artificial emulsion was also tested in groups of guinea-pigs and found to be much slower than in the rat. In these animals approximately 62 p.c. of the total fatty acid injected had been absorbed in 16 hours and small amounts of emulsion were still present 24 hours after the injection. This slower rate of peritoneal absorption in the guinea-pig has also been shown to occur with plasma proteins and whole blood (Courtice and Steinbeck, 1950; Courtice, Harding and Steinbeck, 1953).

In the rat, the rapid rates of removal of injected fat were reflected in increased levels of total fatty acid in the circulating plasma. The increase in plasma fatty acid concentration paralleled the peritoneal absorption curves, the plasma lipids remaining elevated while fat was being rapidly absorbed from the peritoneal cavity. With homologous lymph, the plasma fatty acid concentration reached a peak in the first hour following intraperitoneal injection, while with cat lymph the plasma levels increased throughout the 4-hour post-injection period. The plasma lipids reached their highest concentration during the absorption of the artificial emulsion and maximum concentrations were found about 4–8 hours after injection. In the guinea-pig there was little change in the level of circulating fatty acid, and this reflected the slow rate at which the prepared emulsion was absorbed in this animal. These changes occurring in the plasma total fatty acid concentration are shown in Fig. 2.

It was possible to compare the rates of absorption of injected dye-labelled protein molecules with chylomicron fat in samples of homologous and heterologous fatty chyle. Following injection, there is a diffusion of unlabelled protein and fluid into the peritoneal cavity and the percentage absorption figures for total protein and fluid represent the resultant of the two processes of absorption and diffusion. As diffusion of chylomicrons and labelled protein molecules during the absorption period is negligible, absorption figures for total fatty acid and T-1824 represent closely their true removal rates. The absorption curves for both fat and T-1824 from homologous and heterologous lymph are shown in Fig. 3. No significant difference was found in the rate of removal of these two substances even though their particulate size varies greatly. As was the case with the chylomicron fat, the heterologous protein of cat's thoracic duct lymph was absorbed significantly slower than homologous lymph protein.

The "foreign" effect of heterologous lymph and prepared fat emulsion. The absorption of heterologous plasma from the peritoneal cavity has been shown by Courtice and Steinbeck (1950) to be accompanied by a "foreign" reaction, characterized by varying degrees of increased capillary permeability

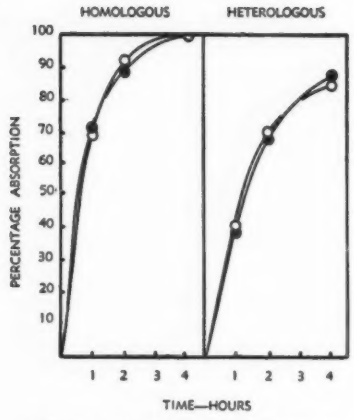

Fig. 3. The absorption of fat and protein in the rat following the intraperitoneal injection of homologous and heterologous fatty chyle, 20 ml./kg. body weight.

O Protein.

● Fat.

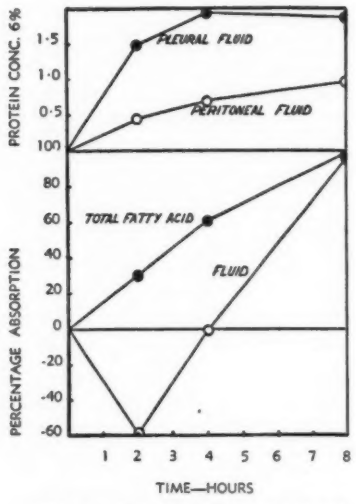

Fig. 4. The absorption of fat and fluid in the rat following the intraperitoneal injection of a prepared fat emulsion, 20 ml./kg. body weight containing 367·9 meq./l. of total fatty acid. The exudation of protein into the peritoneal and pleural fluids is shown in the upper graph.

and exudation of protein and fluid. The "foreign" effect of cat's thoracic duct lymph was compared with that of cat's plasma, and although both these fluids caused exudation of protein and fluid when injected intraperitoneally into rats, the effect was significantly greater with samples of cat plasma. The outpouring of protein and fluid was most pronounced in the first hour following the injection, when exudation of fluid and protein into the peritoneal cavity frequently exceeded its rate of removal. The presence of high concentrations of chylomicron fat appeared to have little effect on the "foreign" reaction as samples of heterologous plasma with a relatively low concentration of chylomicrons were absorbed more slowly than samples of milky chyle (Table 1). Following the intraperitoneal injection of the prepared emulsion in both the rat and the guinea-pig, there was a marked exudation of protein and fluid. In the rat the initially protein-free emulsion contained up to 0·7 gm.p.c. of protein 4 hours after injection and up to 2·7 gm.p.c. 24 hours after injection into the guinea-pig. In the guinea-pig, the fat recovered after 24 hours was clotted and the emulsion had broken. These changes are shown in Fig. 4 where the rate of absorption of total fatty acid and fluid is shown together with the changes in the protein content of the injected emulsion.

TABLE 1.

The absorption of fluid and total protein from the peritoneal cavity of the rat, following the injection of cat's plasma and lymph, 20 ml./kg. Mean results are given for groups of 4 rats at each time interval together with their standard errors.

Sample	1 hour p.c. absorption		2 hours p.c. absorption		4 hours p.c. absorption	
	Fluid	Protein	Fluid	Protein	Fluid	Protein
Plasma 31·4 meq./l	−27±4	5±8	−9±6	27±5	−10±8	29±6
Lymph 75·1 meq./l	13±2	19±4	40±8	41±8	63±4	67±4
Lymph 207·4 meq./l	9±12	32±7	23±7	45±9	62±10	66±9

During the absorption of fatty chyle from the peritoneal cavity of the rat, variable amounts of pleural fluid appeared. This phenomenon has been described previously by Courtice and Steinbeck (1950), occurring during the absorption of heterologous plasma from the peritoneal cavity. They considered the fluid was derived from the injected peritoneal material and was a damming-back effect caused by a bottle-neck along the lymphatic absorbing pathway. In the experiments described here, the largest extravasations of pleural fluid were encountered during the absorption of the prepared emulsion and the heterologous lymph and plasma, whilst homologous lymph and plasma produced minimal amounts of pleural fluid. The pleural fluid found after the intraperitoneal injection of fatty chyle was always milky in appearance, containing large amounts of lipid, and seemed certainly to be derived from the peritoneal material during its absorption. The protein concentration of the pleural fluid was usually higher than that of the peritoneal fluid, but the total fatty acid content was less. Samples of fluid from the pleural cavities of rats obtained 4 to 8 hours after the injection of the prepared emulsion had an average of 1·9 gm.p.c. of protein and 105 meq./l. of total fatty acid.

DISCUSSION.

The very rapid rate at which the injected fat emulsions were removed from the peritoneal cavity stresses the absorptive capacity of the rat's peritoneum. The absorption rates are such as might be expected from known rates of removal of physiological substances such as plasma proteins and red cells (Courtice and Steinbeck, 1950; Courtice, Harding and Steinbeck, 1953; Morris, 1953). The negligible absorption of fixed oil from the peritoneal cavity (Schwartze, 1921) can be appreciated from considerations of the limiting pore size of the absorbing diaphragmatic serosa (Allen, 1951). Emulsified fat, however, provided it remains stable following intraperitoneal injection, provides particles of a size compatible with their transfer across the absorbing peritoneum into the terminal lymphatic lacunae. It is hard to reconcile the results of

Rutenberg *et al.* (1949) with the rapid absorption rates found to occur in the rat and guinea-pig, unless the emulsion used had broken down after injection. Using an emulsion of radioactive iodinized fat containing 10 p.c. of soya bean oil stabilized with Demal, these workers concluded practically all the injected fat remained in the peritoneal cavity for at least 16–17 days after injection. Weyeneth and Calame (1947) and McClendon and Gershon-Cohen (1952), using halogenated fats in colloidal suspension for hepato-splenography in the rabbit and rat, concluded fat absorption from the peritoneal cavity was a process lasting several weeks. If the emulsions used in these experiments were irritant and unstable after introduction, the particles would aggregate and their entrance into the terminal lymphatics would be greatly restricted. The results reported here indicate the significantly slower rates of removal of the prepared artificial emulsion over the physiological emulsion of thoracic duct lymph, even though the state of physical dispersion in each case is similar. The prepared emulsion used in the experiments described was certainly not a bland fluid, as protein and fluid exudation following its injection was considerable. The finding that the relatively large chylomicrons were absorbed at about the same rate as protein molecules suggests that the diaphragmatic absorbing surface offers little restriction to the entry of fat particles, at least those of a size of 0·5 to 1 μ.

The increase in the concentration of total fatty acid in the circulating blood during the absorption phase gave further evidence of the rapid rate of removal of fat from the peritoneal cavity. Intravenous infusions of large volumes of fatty chyle are rapidly removed from the blood stream (Morris, 1954), and in the experiments described here the rate of absorption of fat from the peritoneal cavity was evidently faster than its rate of removal from the blood. The high levels of circulating lipid found during the absorption of the artificial emulsion point to its slower rate of removal from the blood stream. The artificial emulsion is seen to be absorbed much more slowly from the peritoneal cavity than is homologous fatty lymph and is also removed more slowly from the circulating plasma.

Lake, Simmonds and Steinbeck (1953) investigated the nature of the capillary damaging factor present in "foreign" plasma and suggested a heterologous lipoprotein fraction was responsible for altering capillary permeability. The fact that "foreign" cat's thoracic duct lymph produced a significantly smaller "foreign" reaction than did cat's plasma is consistent with there being less of the capillary damaging factor in lymph than plasma. Morris (1954) and Courtice and Morris (1955) found consistently lower levels of lipoprotein in the lymph than in the plasma of various animals they examined. In the cat, the lipoprotein content of the thoracic duct lymph was found to be about 50–60 p.c. of the plasma levels. If the "foreign" plasma reaction is due to a capillary damaging effect caused by heterologous lipoprotein, the presence of large amounts of absorbed fat in particulate form did not appear to enhance

this effect. It is known that the high density lipoprotein spectrum of both plasma and lymph is unchanged during the absorption of fat, and absorbed lipid is carried in the form of chylomicrons. It appears that lipid present in this form plays no significant part in the "foreign" plasma reaction.

SUMMARY.

The peritoneal absorption of homologous and heterologous fatty thoracic duct lymph and of a prepared emulsion of olive oil has been studied in the rat and found to be rapid and complete. Injected at the rate of 20 ml./kg., homologous fatty lymph was absorbed in 3–4 hours, heterologous fatty cat lymph in 5–6 hours, and the prepared emulsion in about 8 hours. During the period of absorption the level of total fatty acid in the plasma increased significantly.

The relative rates of absorption of homologous and heterologous T-1824 labelled lymph protein and chylomicron fat were studied and found to be essentially similar.

Following the intraperitoneal injection of heterologous lymph and the prepared emulsion, there was an exudation of fluid and protein into the peritoneal cavity. The "foreign" reaction observed with cat lymph was significantly less than with cat plasma and was not affected by the presence of large amounts of chylomicron fat.

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THE NEW ZEALAND STRAIN OF OBESE MICE

THEIR RESPONSE TO STILBOESTROL AND TO INSULIN

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During the last decade it has become possible to study the problem of obesity in rats and mice. The literature on experimentally induced and hereditary obesity of rodents has been comprehensively reviewed by Mayer (1953a). Since then Furth (1953) has described a new type of obesity which develops in mice bearing transplants of ACTH secreting pituitary tumours. We have reported on an inbred strain of mice with hereditary obesity (1953) and Mayer and his associates (1955) have reproduced the hypothalamic obesity in mice.

In this paper a detailed account will be given of the natural history of the New Zealand obese mice (NZO) and of some of the metabolic abnormalities characteristic of this strain.

METHODS.

The mice are kept at a temperature of 70±2° F. in thermostatically controlled rooms. The stock diet, given *ad lib.*, consists of wheat grain and a mash of the following composition, bran 35 p.c., pollard 40 p.c., meat meal 20 p.c., bone flour 5 p.c. To each 3 kg., 100 ml. of cod liver oil and 180 gm. of iodised salt are added. The breeders receive wheat grain and a special mash consisting of: wheat germ 200 gm., dried skimmed milk 1250 gm., dried yeast 250 gm., pollard 400 gm., cod liver oil 150 ml., butter 225 gm., iodised salt 100 gm., ferrous sulphate 5·0 gm. In addition, greens are given once weekly to all animals; meat meal and lightly boiled beef liver to the breeders. The mice have always access to water. The experimental animals were weighed once weekly.

Hagedorn-Jensen's method of blood sugar estimation was chosen because it was found to give reproducible results with very small quantities of blood. Samples of 0·02 ml. were taken from a cut in the tail with opsonic pipettes (H. J. Elliott Ltd., Treforest, Glam., U.K.).

Estimation of total lipids in whole carcass or liver was carried out in the following manner: the fresh liver was weighed, ground up with sand and anhydrous sodium sulphate and subsequently extracted with alcohol-ether (3: 1, by vol.) for 20 hours in a Soxhlet apparatus. An aliquot part of the extract was brought to dryness at low temperature and the residue re-extracted with petroleum ether-chloroform (3: 1, by vol.). After filtration the solvents were evaporated and the final residue was weighed. The carcass was kept for 24 hours at a temperature of 0–2° C., cut up finely with scissors and then treated as described for liver.

For the staining of the β cells of the islets of Langerhans a solution of 0·5 gm. of stable fuchsin-aldehyde (Bayer, Leverkusen) in 100 ml. 70 p.c. ethanol containing 0·25 ml. conc. hydrochloric acid was used. This preparation has the advantage of maintaining its staining intensity unaltered for many weeks. Bouin fixed material gives the best results. Oxidation of the sections (3–5 μ) with 0·3 p.c. permanganate in 0·3 p.c. sulphuric acid, followed by bleaching with potassium metabisulfite prior to the staining with fuchsin-aldehyde, is essential. Weigert's haematoxylin was used as nuclear stain, and as counter-stain for the α cells either azophloxin or methyl green in saturated picric acid.

For the staining of lipids Lillie's oil red O procedure, and for glycogen the same author's allochrome method (1951) were used.

EXPERIMENTAL.

The origin of the obese mice.

The New Zealand strain of obese mice (NZO) has been obtained by systematic inbreeding. We started in 1948 from a pair of agouti mice chosen from the mixed stock of a colony which had been brought to the Animal Department of the Otago Medical School in 1930 from the laboratories of the Imperial Cancer Research Fund, Mill Hill, London. The mice of this colony show extreme variation in phenotype. Obesity occurs occasionally and is not related to any coat colour. After 10 generations of brother-sister mating, long after the coat colour had become uniform, obese animals appeared in increasing numbers in the descendants of the two original agoutis. From the 12th generation onwards we selected for the propagation of the strain offspring from animals showing this character. In successive generations the trend for obesity became increasingly pronounced and a short report on metabolic abnormalities observed in mice of the 18th–21st generation was published in 1953 (Bielschowsky and Bielschowsky).

From four other strains developed at the same time, mice of the NZC strain conform best to the normal pattern and have therefore been chosen as controls for NZO mice in some of our investigations. The NZC strain is now in the 33rd generation.

Breeding performance.

In 1953 we were not certain whether it would be possible to maintain the NZO strain. The then rather poor breeding performance has improved in the meantime and we are confident of not losing the strain which is now in its 30th generation.

Since the female mice with hereditary obesity, described by Ingalls, Dickie and Snell (1950), are not fertile (Runner and Gates, 1954), it seems justified to give an account of the fertility of the NZO mice.

Whereas in the past first litters hardly ever survived, currently the great majority develop normally. To illustrate the improvement in breeding performance the following data are given. Of 161 NZO females mated when

8 weeks old 151 produced litters. The number of offspring of 130 mothers is known, the remaining 21 had destroyed some or all of the newborn. From 130 first pregnancies 717 offspring were obtained. Of these 32 were either stillborn or died during the first 24 hours and 37 died before reaching weaning age, i.e., 648 or 89·6 p.c. survived. For 55 second litters the mortality was 7 p.c. The average litter-size was 5·5 and 4·9 respectively.

Although these figures indicate a satisfactory breeding performance it is still greatly inferior to that observed in our NZC strain as shown by a comparison of 50 breeders with 2 or more litters. Whereas 45 of 50 NZC females had the second litter within 19–25 days of the first, only 16 of 50 NZO females had their second litter within this interval. In the other 34 the second parturition took place 26–88 days after the first.

In the NZO females the vagina opens between the 25th and 35th day of life. Vaginal smears taken from 10 virgins in monthly intervals for periods of 10 days from the second to the ninth month of life showed that a regular 4–5 days cycle is seen only occasionally. Smears consisting of cornified cells exclusively were often present for 3 consecutive days. In the ninth month only 5 of the 10 females came into oestrus. These data suggest that the relatively low fertility of the NZO strain is due to the females.

The bodyweight.

During the first month of life there is a great variation in weight between animals born from different mothers irrespective of litter size. Some mice weigh up to 20 gm. when weaned at 28 days, whereas the average weight in other litters is only 12 gm. These striking variations disappear slowly during the succeeding 3–4 months when obesity develops in nearly all animals with the exception of breeding females. Currently, in healthy males 8 months of age bodyweights under 47 gm. hardly ever occur, the majority weighing between 50 and 60 gm. Of 40 females of the same age only two weighed 38 and 41 gm. respectively, all others 44 gm. or more. The weights most frequently found range between 50 and 60 gm. Whereas in earlier generations variations in the degree of obesity were more pronounced and some males reached weights up to 89 gm., at present few surpass 68 gm., and 72 gm. seems to be the maximum. In females in which extremely high weights were never observed, the maximum weight remains at 72 gm.

In the second year of life, especially after the 18th month, many NZO mice lose weight. Very often this is due to disease, and in most instances to the development of tumours. In some animals, however, inflammatory and neoplastic processes could be excluded as causes of the decline in weight.

As already mentioned, repeated gestation in rapid succession delays the onset of obesity, but one or two pregnancies early in life do not have this effect. Of two 8 months old sisters one, a virgin, weighed 58 gm., the other, a

breeder which had her last litter at 6½ months of age, weighed 43 gm. The corresponding figures for another pair were 55½ and 43½ gm. However, once the breeders are separated from the males their weight increases gradually to that of their virgin litter mates.

That the high weights recorded for the great majority of mice of the NZO strain are due to excess body fat will be obvious from Fig. 1, showing adult females of the NZO and the NZC strain. The abdominal girth of the NZO mouse is remarkable, but the extremities and neck have a circumference not strikingly different from that of non-obese mice. In the NZO strain the largest fat deposits are found in the abdomen and the retroperitoneal tissue. The subcutaneous fat tissue, which is increased in thickness above that of normal mice, is more developed in the lower than in the upper half of the body. The total lipid content of a male 6½ months old weighing 53½ gm. was found to be 20·4 gm., i.e., 38·1 p.c. of the bodyweight. Therefore there can be no doubt that NZO mice are in fact obese.

Gonadectomy performed at the age of 4–6 weeks has no effect on bodyweight during the subsequent 2 months. Then follows a period where the castrates gain more rapidly than their intact litter-mates, but the maximum weight reached by the gonadectomized animals is of the same order of magnitude as that of normal NZO mice.

Some morphological and physiological features of NZO mice.

A macroscopically recognizable gall bladder is missing in over 90 p.c. of the animals. The common bile duct emerges directly from the visceral surface of the liver and enters the duodenum approximately 10 mm. below the pylorus.

Frozen sections of the liver show fat in appreciable amounts.

The seminal vesicles (Fig. 2) are unusually large and distended by abundant secretion which, by coagulation, frequently causes formation of foreign bodies in the bladder. These can block the urethra and, if not removed by gentle pressure, produce urinary retention, secondary inflammatory changes, and ultimately the death of the animal.

Of the endocrine glands the pancreas shows the most pronounced changes. The islets of Langerhans are numerous and often unusually large, some reaching giant size. For instance, the ribbon of islet tissue crossing the section depicted in Fig. 3 measures 1·6 mm. in length. When stained with the fuchsin-aldehyde reagent of Gomori (1950) the β cells are seen to occupy not only the centre, but also most of the periphery of the islets (Fig. 4). A well-defined marginal zone formed by α cells is rarely present. These elements lie dispersed in the periphery between the β cells, which represent approximately 90 p.c. of the cell population. The intensity of granulation varies from cell to cell. In some the whole cytoplasm is filled with granules, whereas in others only fine, dust-like material is stainable with fuchsin-aldehyde (Fig. 5). The size of the islets

increases with age. Figs. 6 and 7, taken at the same magnification, depict the typical appearance of the islets before and after onset of obesity. In comparison with islets of mice of other strains, those of the NZO mice show often many blood-channels filled with erythrocytes, which give the islets a kind of trabecular structure (Fig. 8).

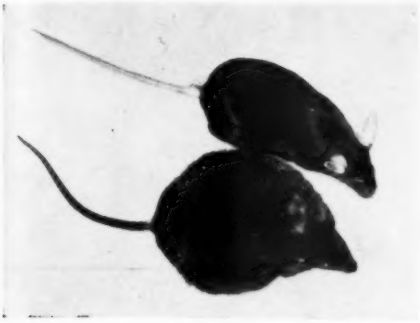

Fig. 1. Two adult females of NZC and NZO strains.

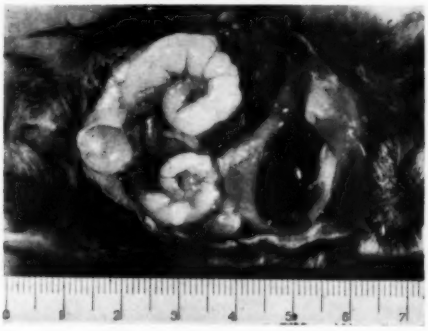

Fig. 2. Seminal vesicles of 8½ months old NZO male.

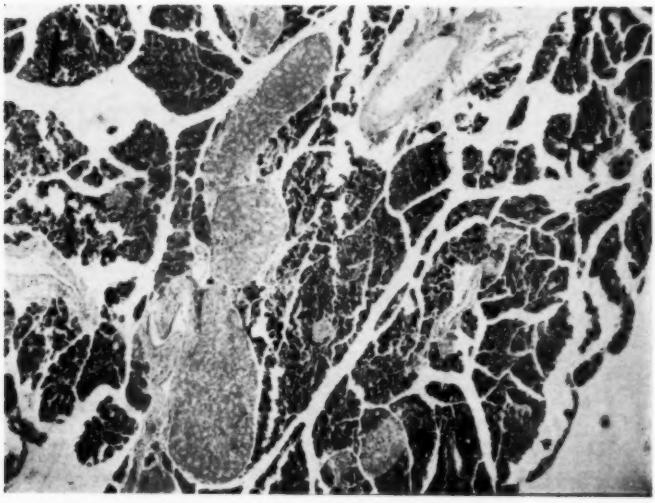

Fig. 3. Pancreas of 13 months old NZO male. H. & E. ×50.

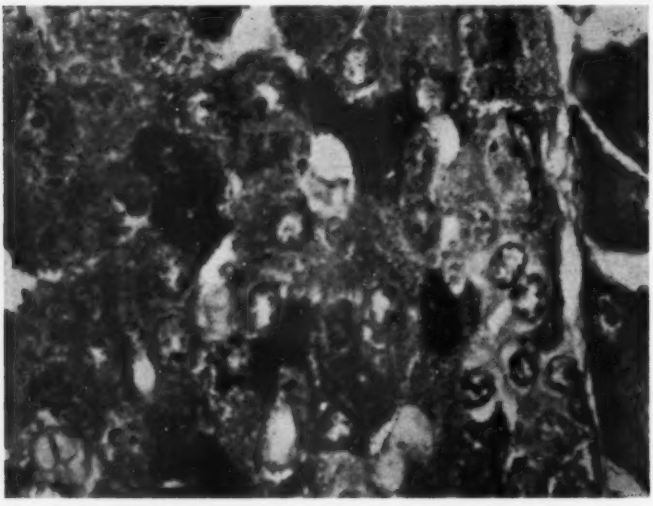

Fig. 4. Detail from islet of Langerhans. The light, not granulated cells in periphery, are α cells, the granulated elements are β cells. (Fuchsin-aldehyde—Weigert H.—Azophloxin.) ×1000.

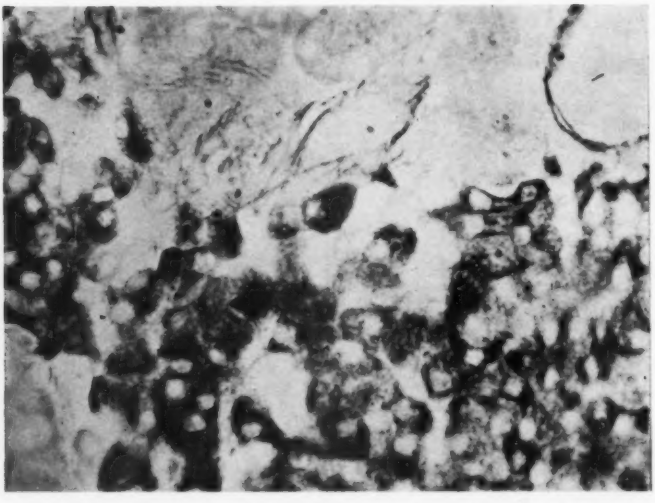

Fig. 5. Appearance of β cells in islets of Langerhans of 6 months old NZO female. (Gomori Fuchsin-aldehyde without counterstain.) ×500.

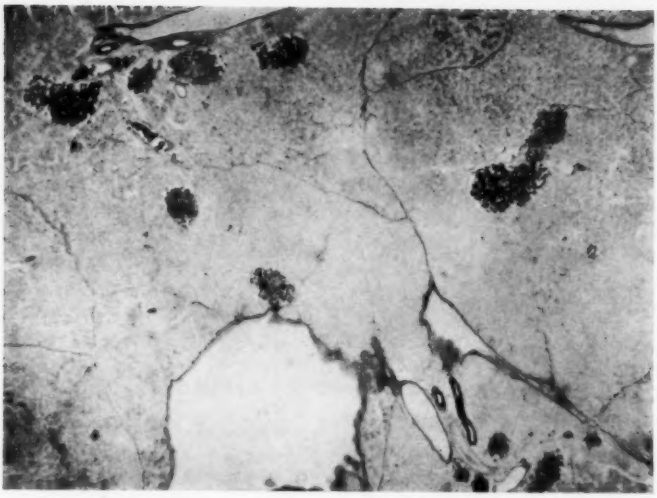

Fig. 6. Pancreas of 1 month old NZO male. (Gomori Fuchsin-aldehyde without counterstain.) ×50.

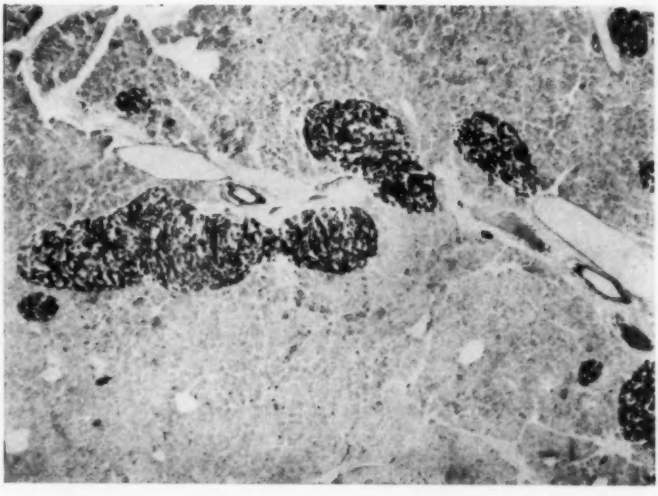

Fig. 7. Pancreas of 6 months old NZO female. (Gomori Fuchsin-aldehyde without counterstain.) ×50.

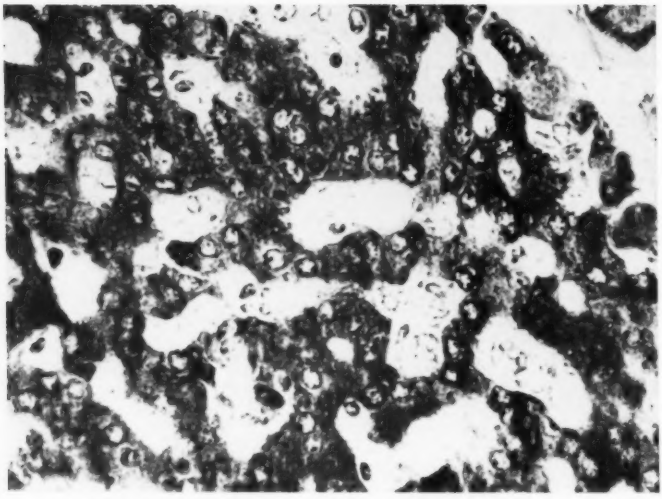

Fig. 8. Islets of Langerhans of 14 months old NZO female showing great vascularity and trabecular arrangement of the β cells. (Fuchsin-aldehyde—Weigert H.—Azophloxin.) ×500.

Fig. 9. Adrenal of 8 months old NZO female. (Oil Red O—Haematoxylin.) ×50.

Fig. 10. Adrenal of 7 months old NZC female. (Oil Red O—Haematoxylin.) ×50.

The adrenals of adult obese NZO are larger than those of NZC mice of similar age. In frozen sections one sees that the amount of stainable lipid is less in the obese, and that the distribution of lipid throughout the cortex is not as even as in the NZC. There is also a slight nodularity noticeable in the adrenal cortex of adult obese mice (Figs. 9 and 10).

In view of the great inactivity recorded by Mayer (1953b) as characteristic for the obese mice of the Jackson Memorial Laboratory, it is necessary to state that our strain shows a different behaviour. Young animals up to 3 months are extraordinarily lively. Older, already obese, animals are somewhat quieter than mice of other strains, and only the very obese ones show a marked inactivity. However, even these can be incited to spurts of great activity by the introduction of another male into their cage, leading to a ferocious fight ending often in death.

Water consumption of NZO mice is greater at all ages than that of NZC mice. In an experiment in which the water intake of 3 months old males was measured, the average daily consumption was 4·5 ml. for the NZO and 2·0 ml. for the NZC mice. Calculated on the basis of 100 gm. bodyweight/day, the values are 11·4 and 7·9 ml. respectively.

The blood sugar.

Elevated blood sugar levels in conjunction with obesity have been reported frequently in man as well as in rodents. A systematic study of the blood sugar level in NZO mice resulted in the following findings:

The blood sugar level in starved and unstarved animals depends partially on the diet offered. If not stated otherwise, all values given in the Tables refer to mice of the NZO and NZC strains kept on the ordinary stock diet. The blood samples were always taken between 12.15 and 12.30 p.m. In NZC mice 3 hours after withdrawal of food the blood sugar ranges from 80 to 125 mg. per 100 ml. In unstarved females no value over 160 mg. p.c. has been found, but higher values have occasionally been observed in unstarved males. In contrast, 3 hours' starvation makes little difference in the case of the NZO mice. After withdrawal of food for 4 to 20 hours quite unusual variations in blood sugar values have been observed. Occasionally there is a rise after 5 hours of starvation, and even 20 hours do not always bring the blood sugar down to normal. At this time the animals are obviously in a bad general condition and we have therefore refrained from pursuing this line of enquiry.

There is a definite trend towards higher blood sugar values from the 4th month of life onward, but in the second year values above 200 mg. p.c. are the exception. Gonadectomy does not seem to alter blood sugar levels significantly (Table 1).

There is, however, one condition under which the blood sugar level remains inside the normal range, in pregnancy. Table 2 gives a comparison of blood

sugar values of pregnant females and their virgin sisters. Remarkably low figures were found during parturition. During lactation the blood sugar rises but does not quite reach that of the non-breeding sisters (Table 3).

To summarize: during the development of the obesity there is a trend towards an elevated blood sugar, but during pregnancy the blood sugar levels

TABLE 1.

Range of blood sugar values in 176 normal NZO mice (mg. per 100 ml.)

Age (months	Number	Sex	mg. p.c.				
			100-120	125-160	165-200	205-240	245-300
1-3	24	♂	1	8	6	7	2
	16	♀	1	8	6	1	0
4-6	31	♂	0	6	11	12	2
	47	♀	2	16	19	10	0
7-12	16	♂	0	5	4	5	2
	22	♀	1	8	4	9	0
13-20	7	♂	2	2	3	0	0
	13	♀	2	8	2	1	0

TABLE 2.

Blood sugar values of 5 pregnant and 3 littering NZO ♀. Controls: virgin litter-mates. Breeder diet (mg. per 100 ml.)

Age (months)	Pregnant mg. p.c.	Control mg. p.c.	Littering mg. p.c.	Control mg. p.c.
3½	120	155	100	195
3½	120	175	70	160
4	125	150		
4½	145	160	70	160
5	75	195		

TABLE 3.

Blood sugar values of 7 lactating NZO ♀. Controls: virgin litter-mates. Breeder diet (mg. per 100 ml.)

Age (months)	Lactating mg. p.c.	Control mg. p.c.
4	120	145
4	135	145
4	155	160
4½	140	150
4½	150	185
4½	150	185
4½	155	160

are normal. A definite correlation between the degree of obesity and hyperglycaemia could not be established. In some animals the blood sugar is remarkably constant over months, but in others it fluctuates widely, independent of gain or loss of weight. Even with blood sugar values above 200 mg. p.c. the urine contains little or no reducing substances. A strongly positive Benedict reaction was rarely obtained; occasionally the formation of a yellow precipitate with a still blue supernatant was observed. During pregnancy the urine is free of sugar.

The effect of stilboestrol.

That some interconnection between hyperglycaemia and obesity exists, in spite of the failure to establish more than an age-dependent trend in normal NZO mice, is supported by experiments in which the effect of stilboestrol was investigated. In order to obtain continuous action, pellets consisting of 2·5 p.c. stilboestrol and 97·5 p.c. cholesterol were implanted subcutaneously. Their weight was 5 to 6 mg. In a few animals where the response to this moderate dose seemed inadequate, a second pellet was injected. Untreated litter-mates served as controls. The response to the action of stilboestrol depended on the age of the animal (Fig. 11). In young 5 to 6 weeks old animals the bodyweight became stationary in the 3rd to 4th week of the experiment. It remained at

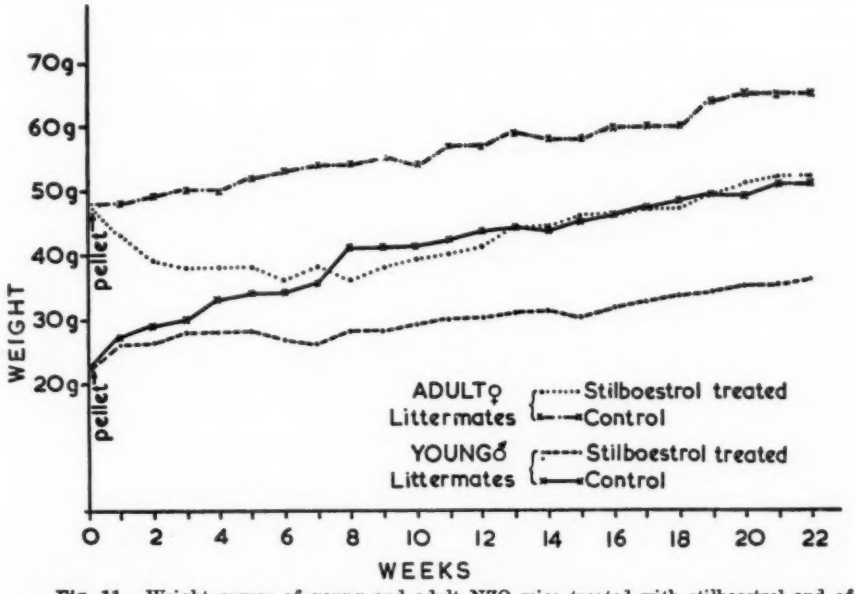

Fig. 11. Weight curves of young and adult NZO mice treated with stilboestrol and of their untreated litter-mates of the same sex.

about the same level up to the 13th week and then rose slowly without reaching that of the untreated litter-mates. The latter showed a continuous gain in weight during the 22 weeks of observation. That the difference in weight in the two groups is mainly due to the amount of fat deposited was shown by an experiment in which total body fat was estimated. A pellet weighing 5·8 mg. (2·5 p.c. stilboestrol) was implanted into a 5 weeks old male. His weight was then 18 gm. and that of the untreated brother 19 gm. Their bodyweights 15 weeks later were 31½ and 43½ gm. respectively, the corresponding body-length 19·2 and 19·6 cm. The carcass of the stilboestrol-treated animal contained 5·30 gm. (14·6 p.c.) fat, and that of the control 13·62 gm. (31·3 p.c.). Subtracting the gm. fat found from the respective bodyweights, one obtains 26·20 gm. for the treated and 29·88 gm. for the normal litter-mate, i.e., about 70 p.c. of the difference in weight is accounted for by fat.

Stilboestrol pellets implanted into adult obese NZO mice induce an immediate drop in bodyweight noticeable already one week after implantation (Fig. 11). Some animals recover their original weight and even show weight gain after 4 to 5 months, but in others the decline is permanent.

With the doses used the effect of stilboestrol on bodyweight lasts for 18 to 25 weeks and even longer in young females.

TABLE 4.

Blood sugar values of 6 NZO ♂ and 6 NZO ♀ treated with stilboestrol. Controls: untreated litter-mates. (mg. per 100 ml.)

Duration of experiment (weeks)	♂ mg. p.c.		Duration of experiment (weeks)	♀ mg. p.c.	
	Treated	Control		Treated	Control
9	95	180	9	110	145
9	110	160	10	130	170
14	120	260	14	125	150
18	125	165	16	100	160
18	110	185	17	125	205
22	120	235	22	160	235

The blood sugar lowering action of stilboestrol (Table 4) appears earlier in young males than in young females. In the former 2 months after implantation the blood sugar level of the stilboestrol-treated animals was considerably lower than in their normal litter-mates. This difference persisted during the following 3 months. In young females, however, the difference in blood sugar values between treated and untreated animals is not so marked with 5 to 6 mg. pellets, but by doubling the dose a pronounced blood sugar lowering effect has been obtained. In spite of the remarkable and immediate reduction of the bodyweight of old animals by stilboestrol, their blood sugar levels are far less affected. A progressive lowering of the originally hyperglycaemic values is only seen in

mice which lose more than 25 p.c. of their weight. Reductions of weight from 60 to 50 gm. are not accompanied by a drop in blood sugar values. To summarize: there is no close correlation between bodyweight and blood sugar level; a dose of stilboestrol which affects bodyweight does not always lower the blood sugar.

It seemed essential to establish whether the stilboestrol effect was due to decreased food intake or to hormonal action. As Table 4 shows, the food intake of

TABLE 5.

Food intake and blood sugar values of NZO ♀ treated with stilboestrol and of their untreated ♀ litter-mates

Stilboestrol treated						Untreated litter-mates					
Weight gm.	Wheat gm./ day	Milk gm./ day	Cal.[1]/ day	Blood sugar mg. p.c.[2] a	b	Weight gm.	Wheat gm./ day	Milk gm./ day	Cal.[1]/ day	Blood sugar mg. p.c.[2] a	b
22	3·4	12·0	18·67	105	165	26	2·5	13·6	16·26	140	215
23	2·2	22·0	19·62	75	130	30	1·4	19·4	15·35	140	255
22	2·6	24·0	22·13	100	125	32	2·7	15·9	18·20	160	300
23	2·4	24·2	21·51	105	110	29	2·2	15·9	16·39	160	285

[1] Calories calculated using the following figures: 362 per 100 gm. wheat (Sherman 1941), and 530 per 100 gm. whole dried milk (McCance and Widdowson, 1942).
[2] Blood sugar values (mg. per 100 ml.); a: before start of the experiment—stock diet; b: at finish of wheat and milk diet.

stilboestrol-treated NZO mice was certainly not less than that of their untreated litter-mates. Semi-starvation can therefore be ruled out as cause of the stilboestrol effect. The experiment was conducted with two litters of 4 females. Into 2 animals of each litter a pellet was implanted when they were 6 weeks old. They were put into individual cages 2½ months later and received for the duration of the experiment a diet consisting of wheat grain and reconstituted full cream milk (1 gm. in 10 ml. water, boiled to avoid clotting). After 24 hours on this regimen food consumption and liquid intake were carefully measured for the subsequent 6 days. We have chosen 4 months old animals for this experiment because at this age NZO mice are still fairly lively and gain weight at a rapid rate. At the end of the experiment the blood sugar had risen considerably in the controls, and to a lesser extent in the pellet-bearing animals, so that the difference between the two groups became even more pronounced.

The pituitaries of the stilboestrol-treated animals were enlarged and hyperaemic. The maximum weights recorded were 11·2 mg. for a male and 11·6 mg. for a female, most weighed 6 to 8 mg. Pituitaries of this size do not produce signs of increased intracranial pressure. A detailed account of the histological changes observed in stilboestrol-treated NZO mice will be given in a separate paper. Only two findings will be mentioned. Appropriately stained sections of liver show more glycogen and less fat than seen in the controls.

The action of insulin.

Resistance to insulin has been observed in various strains of mice, some of which are obese. As already reported in our preliminary communication (1953), the NZO mice withstand doses of insulin not tolerated by mice of the NZC strain which served as control. In this strain doses higher than 0·4 units/kg. of soluble insulin are invariably fatal. The maximum dose tested and tolerated by NZO mice was 20 units/kg. However, some 16 months old males died after injection of 4 units/kg., a non-fatal dose for animals under 1 year. With high doses (2 to 8 units/kg.) of insulin, the blood sugar drops during the first hour. This is followed by a temporary rise to or above pre-injection levels. Then the blood sugar falls again to return slowly to its original value. In order to exclude effects due to glucagon, present in most commercial preparations, we have checked our earlier results using insulin Leo and have observed the same biphasic curves with the Danish insulin (Fig. 12). It seemed important to learn more about the mechanism of blood sugar regulation and to compare the insulin response of stilboestrol-treated NZO mice with that of normal litter-mates and that of NZC mice. For this purpose we have used smaller doses of insulin Leo and have studied particularly the blood sugar changes following the injection of 0·1 unit/kg. In order to minimize variation in resorption due to the differences in subcutaneous fat of obese and non-obese mice, the insulin was injected into the loose subcutaneous tissue below the occiput. Here little fat is present even in very obese animals. In some experiments food was withdrawn immediately before injection, in others 3½ hours previously. In starved as well as in unstarved NZC mice these doses produced always a marked fall of the blood sugar. In mice of the NZO strain they induced a mild hypoglycaemia only in animals bearing a stilboestrol pellet. In starved obese animals there was little change, but in unstarved obese mice a rise in blood sugar followed always the injection of 0·1 unit/kg. of insulin Leo (Fig. 13). In order to see whether this rise, which can last for periods of up to 2 hours, was preceded by a short-lived hypoglycaemic phase, blood samples were taken at 10-minute intervals (Fig. 14). No hypoglycaemic phase was found.

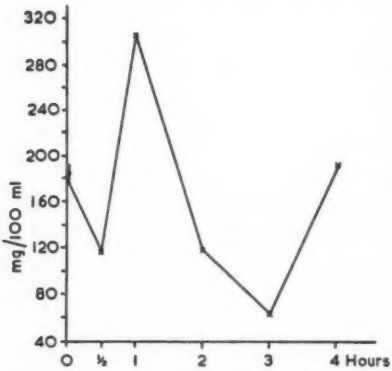

Fig. 12. A typical biphasic blood sugar curve. 3 hours' starvation. 4 units of insulin Leo/kg. (NZO female, 5 months old, 47 gm.).

To summarize: doses of insulin, such as are used in insulin-tolerance tests,

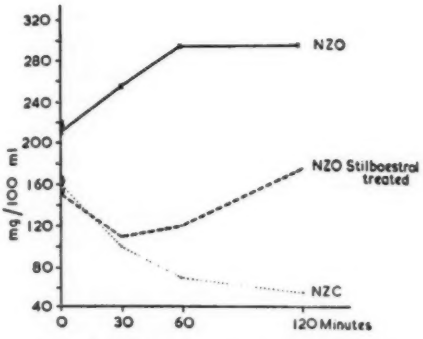

Fig. 13. Blood sugar curves after injection of 0·1 unit of insulin Leo/kg. into an unstarved NZO female (54 gm.), its stilboestrol-treated sister (38 gm.), and a NZC female (26 gm.) (6 months old).

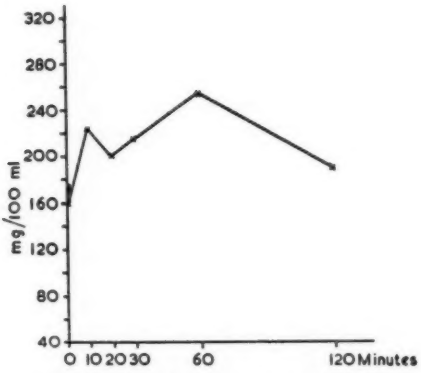

Fig. 14. Blood sugar curve after injection of 0·1 unit of insulin Leo/kg. into an unstarved 7 months old NZO male (55 gm.).

raised the blood sugar level of obese NZO mice provided they had access to food up to the time of injection, but lowered the blood sugar slightly in pellet-bearing animals. The same dose produced a marked hypoglycaemia in NZC mice.

The insulin resistance and still more the paradoxical response of obese NZO mice to insulin raised the question as to the nature of the insulin antagonist. In view of the observation of a remarkable hyperglycaemic response to growth hormone in the hereditary obese mice of the Jackson Memorial Laboratory (Mayer, Andrus and Silides, 1953), we have repeated their procedure using two different growth hormone preparations. One was provided by Dr. Steelman, Armour # 728–076, the other, a Raben-Westermeyer preparation, was supplied by Dr. Loft, of the Frederiksberg Chemical Laboratories, Copenhagen. In the NZO mice 2 mg. given daily for 3 consecutive days failed to induce a constant rise in blood sugar values, nor have we been able so far to raise with somatotropin the blood sugar levels of stilboestrol-treated mice to that of their obese litter-mates.

DISCUSSION.

The type of obesity present in NZO mice is the result of selective inbreeding from a stock in which obesity had been observed in a few animals during previous years. The type of obesity studied by Mayer and his associates arose as a mutation in the V stock of the Jackson Memorial Laboratory. Both belong to the hyperglycaemic hereditary form of obesity, but so marked are the differences between them that one cannot consider the "hereditary obese hyper-

glycaemic syndrome in the mouse" to be a single entity. The NZO strain differs *inter alia* from the obese mice of the Jackson Memorial Laboratory by its fertility, the sensitivity to stilboestrol, and the response to growth hormone. Hyperglycaemia, and especially glycosuria, are less pronounced in our mice (Mayer, Russel, Bates and Dickie, 1953).

A primary failure of the regulation of food intake as seen in hypothalamic obesity cannot be the major causative factor in the development of the obesity in NZO mice, as the food consumption of stilboestrol-treated animals is, if anything, slightly higher than that of their obese litter-mates. One is therefore compelled to consider a metabolic disorder as cause of the abnormal deposition of fat. In 1944, Ogilvie described hypertrophy and hyperplasia of the islets of the pancreas in obese patients. The histological study of the pancreas of NZO mice revealed the presence of giant islets full of granulated β cells, a picture suggesting high insulin production. This assumption is supported by the results of Wrenshall, Andrus and Mayer (1955), who found an increased insulin content in the pancreas of the obese mice of the Jackson Memorial Laboratory. The histological description given in this paper fits the picture seen in our strain as far as the size of the islets and the percentage of α and β cells are concerned. But we have not met frequently with β cells so weakly granulated as to make recognition difficult.

The paradox between hyperglycaemia and high insulin content of the pancreas is only apparent. We have shown that our obese mice react to small doses of insulin with a rise in blood glucose when they are in the absorptive state. The hyperglycaemia of NZO mice appears therefore to be an adaptation mechanism. The insulin tolerance tests demonstrate a rapid hyperglycaemic response to increased levels of insulin in the body not mediated via hypoglycaemia. It is not claimed that hyperinsulinism is the primary metabolic disturbance because, during the first 2 to 3 months of life, the islets of the pancreas do not differ much from those of young mice from non-obese strains.

The paradoxical response to small doses of insulin and the ability to tolerate large doses suggest the presence of a powerful insulin antagonist. The same considerations which led McCullagh (1955) to use successfully oestrogens in acromegalic women for the normalization of diabetic blood sugar curves induced us to try stilboestrol. It is known that growth hormone antagonizes insulin and that pharmacological doses of oestrogen can suppress the production of somatotropin. Whereas there can be no doubt as to the presence of elevated amounts of growth hormone in the active phase of acromegaly, we have no proof that this is true in our mice although stilboestrol normalizes blood sugar levels and prevents excessive fat deposition in young animals. Furthermore, we wondered whether the mechanism by which the excessive fat deposition is prevented in pregnant or growing NZO mice is the same as that which protects animals during the period of growth and during pregnancy against the diabetogenic action of growth hormone (Young, 1951). Despite the circumstantial

evidence in favour of growth hormone as the postulated insulin antagonist, the results we have so far obtained with injected growth hormone have failed to support this hypothesis.

Pregnancy can modify the reaction to elevated amounts of insulin secreted by functional islet cell tumours (Campbell, 1939; Pompen, 1946). Whether this is due to an increased insulin demand during gestation has not yet been established. But if one assumes this to be the case, then the normalization of blood sugar levels and the prevention of obesity in pregnant NZO mice would be the consequence of decreased concentrations of insulin and its antagonist in the circulation.

However, all the evidence so far obtained points to an active principle which we believe to be of pituitary origin. The stilboestrol effect is, in our opinion, due to the action of this oestrogen on the adenohypophysis. A stimulation of the islets of Langerhans as described by Houssay and his collaborators (Houssay, 1951; Rodriguez, 1955) was not observed in our mice.

The histology of the pancreas of untreated obese NZO mice does not indicate hypersecretion of glucagon. One should expect an increase rather than the observed decrease in numbers of α cells if glucagon were responsible for the increased insulin production.

Adrenal cortical function and the possible rôle of ACTH as insulin antagonist have not yet been explored. A slightly increased response to adrenalin has been reported in our preliminary communication (1953).

To summarize: we are convinced that a state of hyperinsulinism exists in our obese mice. Insulin is needed for the transformation of carbohydrates into fat. Since synthesis of fatty acids from carbohydrate is dependent on efficient utilization of hexoses (Chaikoff, 1953), it does not seem that a gross qualitative disturbance in intermediary metabolism is present in our mice. The facts so far established point to an abnormal hormonal regulation as cause of the type of obesity described in this paper.

SUMMARY.

The New Zealand strain of obese mice (NZO) has been obtained by selective inbreeding from a mixed colony.

An account of its fertility and other physiological and morphological features, especially of the islets of Langerhans, has been given.

Stilboestrol corrects some of the metabolic abnormalities of this strain. It lowers the elevated blood sugar and prevents or reduces excessive deposition of fat.

Insulin is tolerated in doses fatal to normal mice. Small amounts of glucagon-free insulin raise the blood sugar of unstarved obese NZO mice.

The opinion is expressed that a state of hyperinsulinism is present in NZO mice and that a pituitary factor, not yet identified, opposes the action of insulin.

Acknowledgments. Our thanks are due to Dr. S. L. Steelman, of the Armour Laboratories, and to Dr. B. H. Loft, of the Frederiksberg Chemical Laboratories, Copenhagen, for generous gifts of growth hormone. We are greatly indebted to Nordisk Insulin Laboratorium for a gift of insulin, and to Dr. Harms, of Bayerwerk, Leverkusen, for a sample of stable fuchsin-aldehyde. We wish to express our thanks also to Miss D. Lindsay, M.Sc., who prepared most of the sections depicted in this paper.

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THE ISOLATION OF KERATINOPHILIC FUNGI, INCLUDING MICROSPORUM GYPSEUM, FROM AUSTRALIAN SOIL[1]

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Emmons (1951), and independently of him, Vanbreuseghem and Van Brussel (1952) were the first to point to the rôle of the soil as a reservoir of pathogenic fungi. Both demonstrated that dermatophytes would grow on sterilized soil as media without added nutrient if suitable conditions of moisture and temperature were met. Vanbreuseghem (1952) put forward the hypothesis that the dermatophytes spend one phase of their life cycle as saprophytes in the soil preserving their capability of infecting human and animal hosts.

Investigations of recent years seem to support these ideas. As well as numerous isolations from the soil of fungi responsible for systemic mycoses, there are a few reports of the finding of dermatophytes in soil. The first report of the discovery of a dermatophyte growing as a saprophyte under natural conditions was that of Muende and Webb (1937). Examining samples taken from different parts of a shed which had housed ringworm-infected calves, they detected a macroscopically visible colony of *Trichophyton gypseum* on dry horse dung. Mandels *et al.* (1948) succeeded in isolating *Microsporum gypseum* from wool fabric which had been subjected to an indoor soil-burial test. However, it does not appear certain that the fungus isolated was growing or originating in the soil. Definite proof of the saprophytic existence in soil of *Microsporum gypseum* was first given by Gordon *et al.* (1952). By means of the membrane filter method they were able to recover several multiseptate spores of *Microsporum gypseum* in a soil sample collected in Tennessee. Other isolations of the same dermatophyte were subsequently reported by Gordon (1953) and by Ajello (1953). Gordon (1953) detected the typical macroconidia of *Microsporum gypseum* together with the spores of *Histoplasma capsulatum* in a soil sample collected from beneath the rear porch of a farmhouse. A certain aliquot of the same soil suspension was streaked upon "Actidione" agar, and after five weeks the dermatophyte was obtained in pure culture. Ajello (1953) made use

[1]This work was supported by a grant from the National Health and Medical Research Council of Australia.

of a different method of isolation which had been described previously by Karling (1946) and by Vanbreuseghem (1952a). He placed short tufts of autoclaved human hair as bait upon the surface of the soil. Hairs overgrown with mycelium were then cultured on a medium containing "Actidione" (cycloheximide), penicillin and streptomycin. Ajello (1953) isolated *Microsporum gypseum* from 37 of 116 soil samples collected in Tennessee and Georgia. A non-pathogenic keratinophilic fungus, *Keratinomyces ajelloi*, was described by Vanbreuseghem (1952b). *Keratinomyces ajelloi* shares the characteristic morphological and biological features of the dermatophytes but is distinguished from them by certain minor features and by its non-pathogenicity. It is presumed to participate in the microbiological destruction of keratinaceous debris in the soil.

MATERIALS AND METHODS.

Soil samples were collected from various parts of New South Wales. For a number of samples we are indebted to the Department of Agriculture of the University of Sydney. Most samples were collected from the upper surface of the site, a few others were taken from depths of 2 to 4 inches. In the procedure of isolating the dermatophyte we followed the technique described by Karling (1946), and by Vanbreuseghem (1952c), and later employed also by Ajello (1953). Sterile Petri dishes were about half filled with the soil sample and moistened with about 20–30 ml. of sterile distilled water. Short fragments of autoclaved human hair were placed upon the surface of the soil. The Petri dishes were then kept at room temperature, and examined from time to time for the development of mycelium on the hair filaments. Hairs overgrown by mycelium were transferred to a selective medium introduced by Georg, Ajello and Gordon (1951) which contained 0·5 mg. of cycloheximide ("Actidione"), 20 units of penicillin and 40 units of streptomycin per ml. of Sabouraud's agar.

RESULTS.

Ninety-six soil samples were examined, 12 (12·5 p.c.) of which yielded cultures of *Microsporum gypseum* and 15 (15·6 p.c.) of which yielded cultures of *Keratinomyces ajelloi*. The average time for visible growth to appear on soil plates was about 30 days in the summer months and 48 in the winter months. There were, however, very wide variations from this average. *Microsporum gypseum* made its appearance as a fine creamy yellow down, covering the hairs (Figs. 1 and 2). On microscopic examination numerous spindle-shaped thick-walled echinulate macroconidia, measuring 40 to 56 by 11·3 to 12·9 microns, and having 5 to 7 septa, were seen; microconidia were also present. Mycelium, growing at first along the hairshaft, later formed conical masses which perforated and eventually broke the hair (Fig. 3). The invaded portion of hair, the mass of mycelium and the macroconidia were deeply stained by cotton-blue dye used in a plastic mountant (Figs. 4 and 5). The hairs became thinner, fragmented, and finally disappeared, and at this stage the fungus died away, leaving the soil surface bare. The mode of attack on living hairs is quite

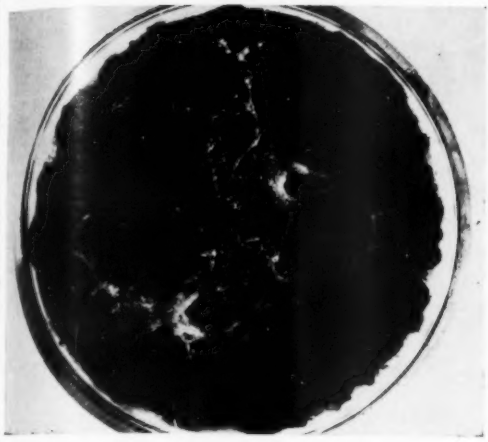

Fig. 1. Petri plate containing soil, showing appearance of *Microsporum gypseum* on human hairs used as bait. (39 days.)

Fig. 2. Petri plate containing soil showing the appearance of *Keratinomyces ajelloi* on human hairs used as bait. (74 days.)

Fig. 3. Hair perforated by keratinophilic fungus. (Soil sample E_1; original magnification ×400.)

Fig. 4. Hair invaded by keratinophilic fungus (mixed culture, including *Microsporum gypseum*). (Soil sample E_{14}; original magnification ×160.)

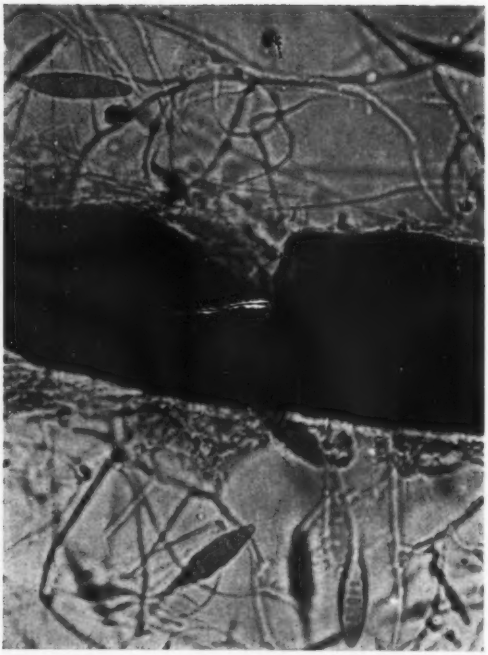

Fig. 5. Hair invaded by *Microsporum gypseum*. (Soil sample B_1; original magnification ×400.)

Fig. 6. Hair divided by growth of *Keratinomyces ajelloi* (×400).

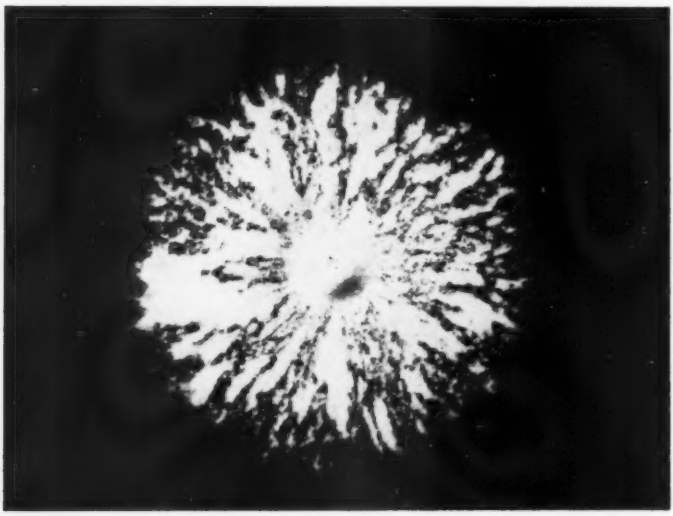

Fig. 7. Colony of *Microsporum gypseum* on Sabouraud's glucose agar containing "Actidione."

different; epilated hairs from cases of ringworm are seen to be surrounded by a mass of small spores with some, usually very little, mycelium. Neither macroconidia nor conical perforations are seen *in vivo*. The selective medium showed two different types of colony; the first had a white central umbo and radiating cream to pale brown strands becoming dendritic towards the periphery, and the reverse of the colony was pale yellow (Fig. 7). The second colony was powdery, darker, almost cinnamon-brown in colour, and the reverse was deep orange at the centre.

Hairs covered with *Keratinomyces ajelloi*, when examined microscopically, showed slender, spindle-shaped smooth thin-walled macroconidia, with 8 to 12 septa (Fig. 6). Colonies on the isolation medium were powdery and dark cinnamon-brown; the reverse was yellow. As this fungus had not been seen here before, cultures were sent to Ajello and to Vanbreuseghem, both of whom confirmed its identification. The latter sent us cultures of strains isolated from soil in Belgium and in the United States.

Other fungi encountered in this survey include species of Fusarium, Sepedonium, Fusidium, Pencillium, and others which have not yet been fully identified, and some of which are keratinophilic.

DISCUSSION.

Until recently, the dermatophytes were regarded as obligatory parasites of man and animals, although the vigour of the saprophytic phase on artificial media had led to the suggestion that they were at least potential saprophytes. It is interesting that the two species of fungi isolated most commonly from soil in this series are the same as those found by Ajello (1953) in Tennessee and Georgia, U.S.A.; and that *Keratinomyces ajelloi* has been grown from soil in Belgium. Zeidberg and Ajello (1954) found that the largest percentage of isolations of *Microsporum gypseum* was from soil samples obtained inside barns, in barnyards and under and around dwellings where animals tended to congregate; that is wherever keratinaceous material was deposited. Thirty of the 96 samples in our series came from sites of this type, and all but one of our 27 isolates came from these thirty samples. Unfortunately most of the samples in our series which came from outlying areas, far from dwellings, had been stored for some months before they were used; this might possibly have affected the viability of the fungi. Whether *Microsporum gypseum* occurs in these sites because keratinaceous debris favours its existence, or because the soil is constantly reinfected by human beings or animals it is impossible to say. It is at least probable that this fungus can exist in the soil for long periods and retain its invasive powers. *Microsporum gypseum* is rare as a cause of ringworm of the scalp. In a series of 122 cases of this disease seen at the Royal North Shore Hospital, 90 (73·8 p.c.) were due to *Microsporum canis* (lanosum),

while only 11 (9 p.c.) were due to *Microsporum gypseum* (Durie, 1956). Figures for the incidence of ringworm due to *Microsporum gypseum* in animals in this country do not seem to be available. Whether human infection arises from contact with soil, or from contact with animals, is not known. The fact suggests strongly, as Vanbreuseghem (1952) has said, that keratinophilic fungi play an important part in the destruction of keratinaceous debris in nature, and that the causation of ringworm in man and animals is a minor and accidental part of their place in the general scheme of things. The method of isolating keratinophilic fungi from soil samples described here is comparatively new; no doubt there is much to be learnt from the examination of larger series of samples and by variations in the methods used.

SUMMARY.

The isolation of *Microsporum gypseum* and of *Keratinomyces ajelloi* from some samples of Australian soil is recorded.

A short preliminary note about this work has been published by Durie and Frey (1955).

Acknowledgments. Our thanks are due to Dr. W. Forbes and Mr. O'Keefe, of the School of Public Health and Tropical Medicine, Sydney, for making the photomicrographs shown in this paper, and also to Miss M. Simpson, of the Royal North Shore Hospital, for the photographs of soil plates.

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POSSIBLE HUMORAL MEDIATORS FOR THE RELEASE OF A.C.T.H. IN STRESS

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Pharmacological experiments involving the systemic administration of "neurohumoral transmitters" and their antagonists have been widely used to study A.C.T.H. release from the pars distalis. Guillemin (1955) has re-evaluated acetyl-choline, adrenaline, nor-adrenaline and histamine as possible mediators of the increased pituitary A.C.T.H. release by stress. His experiments were carried out in rats adapted to the non-specific stressing effect of the antagonists to these substances, so that the administration of the antagonist no longer released A.C.T.H. He then showed that systemic administration of the appropriate blocking drug would prevent the release of A.C.T.H. by the corresponding neurohumoral agent but would not prevent the A.C.TH. release normally induced by systemic stress. Thus his experiments do not support the view that adrenaline, nor-adrenaline, acetyl-choline or histamine are important, indispensable, and sole links in the pituitary activation by stress.

The following experiments were designed to study the same problem by the continuous infusion of various drugs and their antagonists directly into the hypophyseal fossa. This has been achieved by a micro-injector technique. In a previous paper we have discussed the value and use of such a method in the study of the control of hypophyseal function (Rose and Nelson, 1956a).

METHODS.

Operative and micro-injector technique.

The preparation and use of the micro-injector, the detailed technique for the introduction and fixation of a plastic tube into the hypophyseal fossa (H.F.) and the general and endocrinological results of such operative interference have been described (Rose and Nelson, 1956a). In the following experiments we used male rats weighing 180–250 gm. The plastic tube was introduced and fixed in the H.F.; 3 days later the micro-injector was buried in a subcutaneous pocket and immediately connected to the plastic tube. The injector then remained *in situ* and delivered drugs in 0·1–0·2 ml. of buffered solution daily for 8 days.

Index of A.C.T.H. output.

We have used adrenal weights as the index of A.C.T.H. output by the pars distalis. This is a most suitable index if one wishes to assess the A.C.T.H. output over an 8-day period.

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The experiments were carried out using adult rats which would not increase their body weight very much over the short experimental period. Some of our experimental animals did lose weight and we have therefore expressed the adrenal weight in mg. per 100 gm. original rather than final body weight.

It had been our intention to measure the acute pituitary response to the stress of an operation at the conclusion of the experimental period and whilst the infusion of the drug into the H.F. was still proceeding. However, eosinophil counts and adrenal ascorbic acid determinations carried out on a double operated group at the conclusion of the experimental period were low so that it was not possible to detect a further acute A.C.T.H. output following the stress of unilateral adrenalectomy. The acute test was not therefore used in the experimental groups.

Preparation and administration of drugs.

Solutions of yohimbine hydrochloride, procaine hydrochloride, atropine sulphate, Apresoline (1-hydrazinophthalazine hydrochloride), chlorcyclizine, Regitine (C7337), adrenaline hydrochloride, nor-adrenaline, histamine acid phosphate and acetyl-choline were prepared by dissolving the drugs in saline buffered to pH 4·5 with M/250 phosphate. An equimolar concentration of ascorbic acid was included in the solution of adrenaline to prevent oxidation. Eserine sulphate was added to the solution of acetyl-choline to make the final eserine concentration 0·1 p.c. All these drugs were sterilized by heating to 80° C. for 15 minutes after they had been put in the micro-injector. The drugs were delivered continuously and directly to the H.F. in 0·1–0·2 ml. of fluid daily for 8 days. By varying the concentration of the drug and the volume injected *per diem*, a range of doses (0·2–3·5 mg./day) could be achieved.

Experimental design.

In Table 1 the treatment combinations given to the various groups of rats are shown. The following groups may be described: Group A, normal controls; group B, 3 days after implantation of tube in H.F.; group C, 11 days after implantation of tube in H.F.; group D, 11 days after implantation of tube in H.F. and 8 days after the implantation of the micro-injector (double-operated group); group E–N, double-operated group receiving various drugs by the injector technique into the H.F. for 8 days.

At the conclusion of the experimental period the rats were killed by exsanguination whilst under ether anaesthetic and the adrenals removed and weighed. The positions of the plastic tube and the side hole in it from which the drug was delivered were checked to make sure they were correctly placed in the H.F.

RESULTS.

Table 1 gives the results of the adrenal weights (expressed as mg. per 100 gm. initial bodyweight) in normal (group A), control operated (groups B, C, D) and various double operated groups treated with drug infusion into the H.F. (group E to N).

Implantation of a tube in the H.F. for 3 days constitutes a stress and the adrenals in this group are significantly larger than normal (compare group B with A). With a tube in the H.F. for 11 days the adrenals show no further enlargement (compare group C with B). The additional stress of carrying a micro-injector weighing 15 gm. subcutaneously for the last 8 days of the operative period results in a further increase of adrenal size (compare group D with

TABLE 1.

Effect of operative and drug treatments on adrenal weight.

Group	Treatment	No. of observations	Adrenal weight (mg./100 gm.)
A	Normal	12	15·9±2·8*
B	Tube in H.F. for 3 days	16	20·8±1·3
C	Tube in H.F. for 11 days	8	21·0±2·3
D	Tube in H.F. for 11 days and micro-injector subcutaneously for 8 days	27	25·4±2·2
E	Adrenaline into H.F.	8	22·2±4·2
F	Nor-adrenaline into H.F.	6	23·8±5·1
G	Histamine into H.F.	6	24·7±5·7
H	Acetyl-choline into H.F.	6	25·6±4·1
I	Procaine into H.F.	9	25·7±6·01
J	Yohimbine into H.F.	8	23·2±4·48
K	Atropine into H.F.	5	23·9±5·4
L	Regitine into H.F.	5	26·7±6·4
M	Apresoline into H.F.	5	24·6±4·6
N	Chlorcyclizine into H.F.	6	25·2±5·1

* Standard duration.

C). The results show that operative interference to the pars distalis and the presence of a plastic tube in the gland does not prevent the increased release of A.C.T.H. under stress conditions. To assess the effect of a drug infused into the H.F. or A.C.T.H. output, the adrenals of that group must be compared with those of group D.

Continuous infusion into the H.F. for 8 days of adrenaline, nor-adrenaline, histamine or acetyl-choline (dose range for these drugs 0·4–1 mg. *per diem*) did not produce a continued increased A.C.T.H. production with larger adrenals (compare group D with E-H). Continuous infusion into the H.F. for 8 days of procaine (0·8–3·5 mg. *per diem*), yohimbine (0·8–2 mg. *per diem*), atropine (0·6–1 mg. *per diem*), Regitine (0·6–1 mg. *per diem*), Apresoline (0·2–0·7 mg. *per diem*) or chlorcyclizine (0·6–1 mg. *per diem*) did not prevent the usual increased A.C.T.H. release and consequent adrenal enlargement which accompanies the stress of the double operation (compare group D with I-N).

DISCUSSION.

There have been many papers and reviews concerning the regulation of A.C.T.H. secretion under basal and stress conditions and recently the subject has been carefully and critically reviewed by Harris (1955). Three main factors seem to be involved: (1) Inhibitory effect of adrenocortical steroids. (2) Hypothalamic drive either by alleged secretory nerves supplying the pars distalis or by humoral substances reaching the gland via the hypophyseal portal circulation. (3) Systemic drive by humoral substances including adrenaline reaching the pars distalis directly or through their action on the hypothalamus. We have been concerned in the investigation of the latter two mechanisms in these experi-

ments. The micro-injector technique has been applied to this problem and the advantages with its use have been previously discussed (Rose and Nelson, 1956a). When positive results are obtained with systemic administration of "neuro-humoral transmitters" and their antagonists, the question always arises as to the primary site and mode of action and the influence of side effects on this response. When a negative result is obtained it may be due to the fact that the drug has not reached the proposed site of action in adequate concentrations to be effective. If the hypothalamus controls the pars distalis by humoral factors which must be delivered directly to the gland *via* the portal circulation, one might logically infer that direct infusion into the gland would be the desired experimental technique.

We have infused adrenaline, nor-adrenaline, acetyl-choline, histamine, their antagonists and local anaesthetics into the H.F. In no case did we find a larger or less output of A.C.T.H. by the pars distalis. Previous work has shown that drugs infused into the H.F. can influence the activity of the pars distalis. Thus we have shown that procaine and yohimbine can inhibit the increased T.S.H. output of the gland which normally occurs with the administration of propyl thiouracil (Rose and Nelson, 1956a), and we have also shown that hydrocortisone acts directly at the pars distalis to inhibit A.C.T.H. production (Rose and Nelson, 1956b). Moreover, a histological study of the hypophyseal-portal circulation, after Indian ink injection, shows the connective tissue around the implanted tube to be supplied by this circulation so that the special vascular arrangements would assist a small local continuous application of a drug to influence the entire pars distalis. It is difficult to know whether the dose of the blocking drug we give does in fact have the appropriate local pharmacological effect. This is especially important and critical when negative results are obtained. For this reason relatively large doses of drugs were administered —in many cases a systemic and local pharmacological effect could be expected. Thus the dose (0·4–1 mg. *per diem*) of adrenaline, nor-adrenaline, histamine and acetyl-choline were such that one might well expect a systemic as well as a local pharmacological effect. Moreover, with these drugs we expected a possible increased A.C.T.H. output and only a small volume of pituitary tissue would be necessary to provide the increased A.C.T.H. discharge. With blocking drugs it is probably necessary to inhibit most of the pituitary tissue in order to obtain a measurable effect. The dose of yohimbine and procaine used were in the same range as those which blocked the increased T.S.H. output which occurs with propyl thiouracil administration (Rose and Nelson, 1956a). The dose of atropine produced a continued dilation of the pupils during the course of the experiment. The dose of Regitine and chlorcyclizine used was the experimental maximum because larger doses given into the H.F. resulted in the death of most of the animals. If positive results had been obtained the dose of drug would have been reduced until a suitable dose was chosen which would produce

a local pharmacological effect in the pars distalis without an appreciable effect on the general metabolic status.

Our results with this new technique do not support the view that adrenaline, nor-adrenaline, acetyl-choline or histamine are the indispensable and sole links between the hypothalamus and pituitary in the reaction to stress. Moreover, the negative results obtained with procaine and yohimbine, which can block nerve conduction, do not support the notion of secretory fibres supplying the pars distalis. It is of interest to note that the pituitary transplanted to a site away from the hypothalamus showed no secretory activity as far as gonadrophic, growth and thyroid stimulating hormone was concerned, but could still partially maintain adrenocortical function (Westman and Jacobsohn, 1940; McDermott *et al.*, 1950; Cheng *et al.*, 1949; Fortier, 1951).

There are two additional points which must be kept in mind when interpreting our results: (1) A mechanism may exist which will stimulate A.C.T.H. production when the animal is stressed. If this is not the sole mechanism then its removal or inactivation would not necessarily prevent increased A.C.T.H. output with the appropriate stimulus. Thus adrenaline and histamine can both liberate increased A.C.T.H. discharge and this may well be one of the normal ways in which the response occurs. However, administration of the appropriate blocking drug does not inhibit the increased discharge of A.C.T.H. over a long period, as shown in our experiments and in the acute experiments of Guillemin (1955). (2) Acute mechanisms for stimulating A.C.T.H. release may be quite separate and different from those concerned with chronic increased A.C.T.H. output. Thus it is well known that adrenaline and histamine can acutely stimulate A.C.T.H. output, but we have shown in our experiments that continued administration of these drugs does not lead to continued increased A.C.T.H. output.

SUMMARY.

Using a micro-injector technique, we have infused drugs continuously for 8 days directly into the hypophyseal fossa of rats. Under these conditions it proved impossible to alter significantly the A.C.T.H. release by the administration of adrenaline, nor-adrenaline, acetyl-choline, histamine, atropine, Regitine, Apresoline, procaine and yohimbine. These experiments do not support the view that adrenaline, nor-adrenaline, acetyl-choline or histamine are indispensable or important factors acting on the pars distalis for the continued increased A.C.T.H. discharge which accompanies chronic stress.

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THE REMOVAL OF IRON FROM HAEMINS

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A considerable number of methods of removing the iron atom of haemins has been devised. These methods have been listed by Fischer and Orth (1937), by Lemberg (1954), and by Lemberg and Legge (1949). In all methods the iron is reduced to the ferrous state when it can be removed under acid conditions. For instance, Warburg and Negelein (1932) have used ferrous acetate-hydrochloric acid, Hamsik (1931) has used stannous chloride-hydrochloric acid, Grinstein (1947) ferrous sulphate-hydrochloric acid, and Paul (1950) pyruvic acid.

The ferrous acetate-HCl method of Warburg and Negelein (1932) has been widely favoured and has been used in this laboratory until recently. However, this method is not easily standardized, mainly because ferrous acetate must be prepared and added to the haemin solution under strictly oxygen-free conditions. Lemberg, Bloomfield, Caiger and Lockwood (1955) have recently preferred the ferrous sulphate-HCl method. Highly purified crystalline ferrous sulphate is a common reagent and when in solution in hydrochloric acid autoxidizes in air much less rapidly than does ferrous acetate. Solutions can therefore readily be prepared in which the ferrous ion concentration is known precisely.

Although the ferrous sulphate-HCl method as used by Lemberg *et al.* (1955) has generally given good results with haemin *a* preparations from ox heart, some difficulties have been found, particularly with haemin preparations derived from other sources. In order to overcome these difficulties a more detailed study of the factors governing the removal of iron from protohaemin and haemin *a* by ferrous sulphate-HCl has been made and is described in the present paper. Some applications of the procedure arising from this study are also given.

EXPERIMENTAL.

Reagents and de-ironing procedure. Haemin solutions in glacial acetic acid were prepared containing 0·5 μ mole haemin per 0·2 ml. solution; in the

[1]This work has been supported by grants from the National Health and Medical Research Council of Australia.

case of protohaemin solutions 0·02 ml. pyridine per 0·2 ml. were needed for solution of the haemin. Crystalline protohaemin was prepared from blood by the method of Schalfejew (Fischer, 1941). Crude haemin *a* was prepared from ox heart according to the method of Lemberg, Morell, Lockwood, Stewart and Bloomfield (1955) to the stage of its first solution in ether. Pure haemin *a* was prepared from highly purified porphyrin *a* by the method of Lemberg and Stewart (1955).

Ferrous sulphate solutions in concentrated HCl were prepared immediately before use. If the solution was required for more than a few minutes it was found necessary to keep it oxygen-free under carbon dioxide. Throughout this paper the concentration of ferrous sulphate is given relative to that of the haemin as the molar ratio.

The procedure used for the removal of iron was as follows (see last page for modified method). 0.2 ml. haemin solution, any other reagents except ferrous sulphate-HCl, and glacial acetic acid to give a final volume of 5·5 ml. were added to a test tube. A stream of carbon dioxide was applied. 0·3 ml. ferrous sulphate solution in HCl was added at zero time. The reaction was stopped by tipping the mixture into a separating funnel containing ether and sufficient dilute sodium acetate solution to bring the pH above 3. The ethereal solution of porphyrin plus haemin (if incompletely de-ironed) was washed several times with water.

The porphyrins were separated from unsplit haemin by acid extraction, using 5 p.c. (w/v.) HCl for protoporphyrin and 20 p.c. (w/v.) HCl for porphyrin *a*. The porphyrin content was determined spectrophotometrically by measurement of the acid porphyrin solution at the maxima of bands I (protoporphyrin, 595 mμ; porphyrin *a*, 616 mμ).

Investigation of De-Ironing Procedure.

Effect of ferrous sulphate concentration. The molar ratio of inorganic iron to haemin iron was varied from 480 : 1 to 10 : 1 for protohaemin and from 20 : 1 to 1 : 1 for haemin *a* by varying the concentration of ferrous sulphate in the concentrated HCl. The results for ratios of 80 : 1 and lower are given in Fig. 1. Molar ratios of 480 : 1 and 240 : 1 applied to protohaemin showed that a large excess of ferrous sulphate did not destroy protoporphyrin. The results in Fig. 1 demonstrate that haemin *a* is much more readily de-ironed than protohaemin. This finding suggested that porphyrin *a* could be prepared relatively free from protohaemin by de-ironing at low molar ratios of inorganic to haemin iron. In Fig. 2 are shown the results obtained using a crude mixture of protohaemin and haemin *a* prepared from ox heart. It can be seen that in two minutes, under the conditions used, approximately 100 p.c. of the haemin *a* and only a small percentage of the protohaemin have been de-ironed. This selective de-ironing technique is not at the moment of much practical application

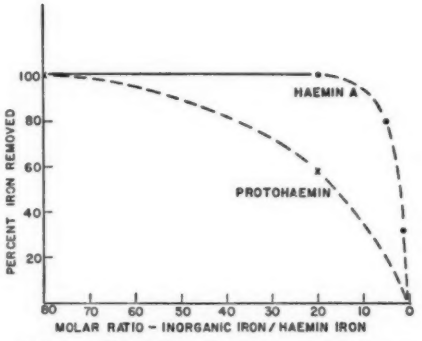

Fig. 1. The effect of ferrous sulphate concentration on the rate of iron removal from protohaemin (X———X) and haemin *a* (O———O). Room temperature. Reaction time 5 min. Ferrous sulphate added in conc. HCl (final HCl concentration =1·9 p.c. (w/v.)).

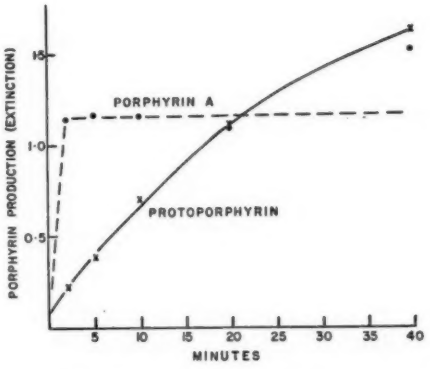

Fig. 2. Selective iron removal. For method of iron removal see EXPERIMENTAL. Molar ratio of inorganic to haemin iron=4·0. Room temperature. HCl concentration as for Fig. 1.

in the preparation of purified porphyrin *a*, however, because protoporphyrin can be efficiently extracted from ethereal solutions of the porphyrins by 4 p.c. (w/v.) HCl. Should it be desired not to use HCl in the purification, selective de-ironing may be of some importance. The principle of selective de-ironing may be useful in other problems.

The effect of water concentration. In Fig. 3 is shown the effect of water concentration on the rate of iron removal by the ferrous sulphate-HCl method. The slope of these curves demonstrates the marked effect of water concentration on the rate of reaction. The points at 0 and 100 p.c. iron removal are not comparable in the two curves since they are determined by other conditions such as time of reaction and ferrous sulphate concentration. From Fig. 3 (curve for 22° C.) it can be seen that a decrease in the water concentration from 10 to 8 p.c. doubles the rate of reaction. The curve for the reaction at 80° C. also shows that under the conditions used by Lemberg and Stewart (1955) the water concentration is critical.

It is therefore of considerable importance in standardizing the method of iron removal to keep the water concentration both low and constant. To this end it has been found desirable to treat haemin solutions in ether (and particularly solvents miscible with water) by evaporating to dryness under reduced pressure before dissolving in glacial acetic acid or acetic acid-pyridine.

The effect of HCl concentration. From the data in Fig. 4 it is suggested that the HCl concentration in the reaction mixture needs to be in the range 1·2–2·0 p.c. (w/v.) for best results. Increasing the HCl concentration above

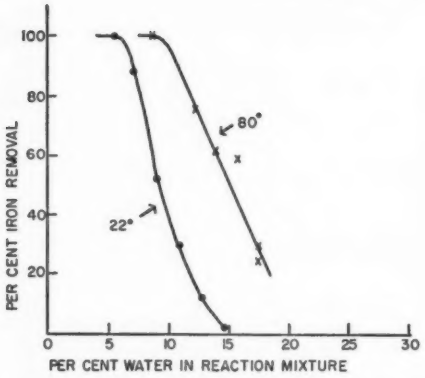

Fig. 3. Effect of water concentration on rate of removal of iron from protohaemin. For method see EXPERIMENTAL. For 22°, reaction time=5 minutes, molar ratio of inorganic to haemin iron=160: 1. For 80°, reaction time=30 seconds, molar ratio=18: 1. For both curves HCl concentration was as for Fig. 1.

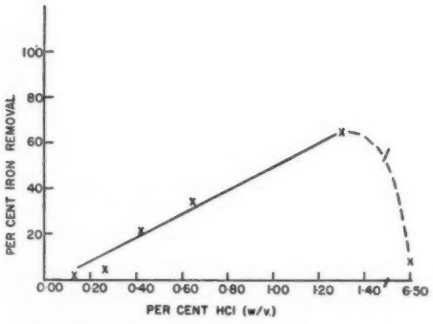

Fig. 4. Effect of HCl concentration on rate of removal of iron from protohaemin. For method see EXPERIMENTAL. Room temperature. Reaction time 5 minutes. Molar ratio of inorganic to haemin iron=80: 1.

this range reduced the rate of reaction because of the water content of the HCl (see Fig. 4).

The effect of temperature. Lemberg *et al.* (1955) de-ironed haemin *a* preparations using the ferrous sulphate-HCl method at 60–80° C. In the present study, however, it was found that by suitably varying the concentrations of reagents complete removal of iron from protohaemin and haemin *a* could be achieved in a few minutes at room temperature. Measurements of reaction rates at 22°, 50° and 80° C. gave an approximate Q_{10} of 1·8.

The finding that iron removal could be carried out at room temperature suggested to us the means whereby the problem of the origins of porphyrins *aα* and *aβ* could be solved. Lemberg and Stewart (1955) found that highly purified porphyrin *a* obtained from ox heart could be converted by treatment with HCl to two forms of porphyrin *a* which they called porphyrins *aα* and *aβ*. Porphyrin *aα* was found to be partially converted to the *aβ* form on standing in aqueous HCl at room temperature. Porphyrin *aβ* could be completely converted to the *aα* form when heated to 85° C. in aqueous HCl. Since the iron-removing procedure used by Lemberg and Stewart involved heating the mixture in aqueous HCl to 60–80° C. and porphyrin *aβ* was unstable under these conditions, it could not be decided whether crude haemin *a* was related to one or the other of these forms or to a mixture of the two.

This point has now been re-investigated using the technique of iron removal at room temperature described in this paper. One of the characteristics of porphyrin *aβ* is that it is extracted from ether by 5 p.c. HCl whereas the *aα*

form requires 20 p.c. HCl for extraction. When the iron was removed from crude preparations of haemin *a* by this procedure, no detectable amount (less than 5 p.c.) of porphyrin *a* was extracted by 5 p.c. HCl after thorough removal of protoporphyrin with 3 p.c. HCl.

This result shows that no haemin corresponding to porphyrin $a\beta$ was present in crude haemin *a*. Moreover, the mild conditions of iron removal used in the present work suggest that crude haemin *a* is the iron complex of porphyrin a_A. This finding lends very considerable support to the thesis, supported by Lemberg and Stewart (1955), that the haematin prosthetic groups of cytochromes a_3 and *a* are identical.

General Application of De-ironing Procedure.

The method of de-ironing haemins described in this paper has been applied to protohaemin and haemin *a* extracted from ox heart and calf liver. For routine preparations of porphyrin *a* from ox heart mince, using the method of Lemberg, Morell, Lockwood, Stewart and Bloomfield (1955), to obtain the haemins in solution in ether, 160 moles ferrous sulphate per mole of haemin applied at room temperature for 5 minutes (HCl concentration 1·7 p.c. (w/v.), water concentration 5 p.c.) gives virtually complete de-ironing of both haemin *a* and protohaemin. Thorough extraction of the porphyrin solution with 8 p.c. HCl to remove protoporphyrin and any cryptoporphyrin yields porphyrin *a* for which the ratio, bands III/IV=1·8–1·95. Chromatography on a cellulose column, according to Lemberg *et al.*, gives pure porphyrin *a* of ratio bands III/IV=2·0–2·1. Experiment has shown that the rate of de-ironing of crude haemins, extracted as described above from ox heart mince, is approximately the same as for pure protohaemin.

On the other hand, haemins extracted from calf liver in five preparations have not been completely de-ironed either by the usual conditions or when the ferrous sulphate-haem ratio was increased to 320 : 1 and the time increased from five to fifteen minutes. Amounts of haemin have always remained unsplit and are sufficient to cause very low ratios (1·0–1·3) of band III/IV for porphyrin *a* after extraction of protoporphyrin and any cryptoporphyrin. In one experiment the percentage of the total haemin (protohaemin+haemin *a*) remaining unsplit was 7 p.c. This was of the same order as the amount of porphyrin *a* produced, and hence greatly affected its spectral purity. From the unsplit haemin protohaemochrome, but not haemochrome *a*, could be formed. The rate of de-ironing of protohaemin obtained from calf liver has been found to be of the same order as that for pure protohaemin or haemin from ox heart mince. Doubling the time of de-ironing did not significantly affect the proportion unsplit. More stringent conditions of de-ironing in which the water content of the reaction mixture was greatly reduced and the HCl concentration increased by using dry HCl in glacial acetic acid (HCl concentration approximately 10 p.c.

(w/v.)), also gave low ratios due to alteration of the porphyrin *a*. In the present study complete de-ironing of calf liver haemin has not been obtained. The low ratios of porphyrin *a* found by Lemberg, Stewart and Bloomfield (1955) for ox liver, and possibly ox kidney and cow udder, are probably also due largely to incomplete de-ironing.

Certain experiments not reported here have indicated the instability of haem *a* towards hydrochloric acid in the presence of glacial acetic acid. The standard procedure for de-ironing has therefore been modified, using an HCl concentration of 0·8 p.c. (w/v.) instead of 1·7 p.c. (w/v.) The lower water concentration, 2·3 p.c. as against 5 p.c., more than compensates for this in terms of rate of de-ironing. In the modified procedure 10·0 ml. glacial acetic acid are used to dissolve 1 μ mole haemin, 160 μ moles ferrous sulphate are added per μ mole haemin, and the ferrous sulphate is always prepared as a 20 p.c. solution in concentrated HCl.

SUMMARY.

Various factors affecting the efficiency of the ferrous sulphate-HCl method of removing iron from haemins have been systematically investigated. The type of haemin, the HCl concentration, water concentration and the ratio of inorganic iron to haemin iron all have marked effects on the rate of iron removal. It has further been shown that under suitable conditions the reaction can be quickly and efficiently carried out at room temperature.

Haemin *a* loses its iron atom much more readily than protohaemin. This finding has been developed to demonstrate the selective removal of iron from haemin *a* in mixtures with protohaemin. Studies involving the removal of iron from haemin *a* at room temperature have shown that porphyrin a_a is the porphyrin corresponding to haemin *a*. This is further evidence that cytochromes a_3 and *a* have identical prosthetic groups.

Application of the above method of de-ironing to crude haemin solutions from ox heart and calf liver has been described.

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WATER SOLUBLE COMPOUNDS (NON-KERATINS) ASSOCIATED WITH THE SKIN FLAKES OF THE HUMAN SCALP

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Of the mammalian skin flakes in general, none have been given the attention which the skin flakes of the human scalp receive. Such attention is marked when human scalp skin flakes become readily visible and are referred to as dandruff. However, little is known about the composition of the skin flakes found on hair in spite of the notoriety they enjoy. The present communication deals with their water soluble constituents.

METHODS.

As in other mammals (Bolliger and Gross, 1954), these flakes usually adhere to the hair. They are bound to the fibre by the sebum secreted from the underlying skin. They can readily be released by immersing the cut hair in ethyl or petrol ether. From 0·5 to 2 gm. of flakes could be obtained by this technique from 100 gm. of hair from normals or from patients not suffering from seborrhea. Flakes may also be collected from the scalp and uncut hair, and in several instances this was done by rinsing the head hairs of cadavers or living persons with ether and combing off the flakes.

Two grams of dandruff were extracted four times with 30 ml. of boiling water for a period of approximately one hour each. The combined extracts were concentrated to 20 ml. The aqueous extract was divided into four fractions as was done in the work on pigeon feathers (Gross, 1955). While the concentrated extract was kept in the refrigerator a precipitate formed (Fraction A). Polypeptides present were then precipitated with trichloracetic acid (Fraction B). Trichloracetic acid was then removed with ether. The precipitate obtained after the addition of 9 volumes of absolute alcohol represented Fraction C. The supernatant alcohol-water solution was concentrated *in vacuo* to a small volume (Fraction D).

Filter paper chromatography and quantitative methods were those used previously (Bolliger and Gross, 1952, 1952a; Gross, 1955).

RESULTS.

Uric acid and guanine were identified by paper chromatography as the main constituents of Fraction A. Fraction B consisted of a polypeptide which,

[1]Working under a grant from the National Health and Medical Research Council.

on hydrolysis, was found to contain the following 14 amino acids as identified by paper chromatography: Aspartic and glutamic acids, serine, glycine, threonine, alanine, leucine, valine, proline, cystine, tyrosine, phenylalanine, arginine and lysine.

Fraction C was characterized by the presence of carbohydrates and gave a blue reaction with iodine, suggesting the presence of polysaccharides. After hydrolysis glucose, arabinose and xylose could be identified with certainty. Rhamnose, ribose and glucuronic acid seemed to be present in small amounts.

Fraction D contained mainly free amino acids. The following were identified: Aspartic and glutamic acids, serine, glycine, threonine, alanine, leucine, valine, proline, cystine, tyrosine, phenylalanine, arginine, histidine, and lysine.

Average quantitative results and ranges in (a) normals, (b) hospital patients without, and (c) hospital patients with renal involvement are presented in Table 1. In three normals the total nitrogen content ranged from 1227 to 1316 mg. p.c. In five hospital patients similar values were encountered, in others values rose up to 1831 mg. p.c. Still higher values were yielded by five patients with varying degrees of renal insufficiency. The highest total nitrogen value (2897 mg. p.c.) was obtained from a female dying of uraemia.

In groups (a) and (b) urea nitrogen varied between 28 and 42 mg. p.c. The patient dying of uraemia had 383 mg. p.c. urea nitrogen in her aqueous skin flake extract. Ammonia nitrogen was in this patient only one-third of that of urea nitrogen, while in all other specimens examined ammonia nitrogen was higher than urea nitrogen.

For the uric acid determination Folin's "direct" method had to be used because of the small amounts of material available. Results varied rather widely from 10 to 43 mg. p.c. In a girl who died of leukaemia it was 56 mg. p.c.

The dandruff of the patient dying of uraemia contained 473 mg. p.c. of free amino acid nitrogen, otherwise this varied between 119 and 326 mg. p.c. It rose to between 200 and 461 mg. p.c. after hydrolysis. These estimations were carried out on the extract deproteinised by tungstic acid and do not include the peptide nitrogen also present in the non-deproteinized extract. The types of amino acids present were revealed by paper chromatography as mentioned earlier.

Before hydrolysis reducing substances expressed as glucose varied between 362 and 996 mg. p.c. On hydrolysis they rose to between 450 and 1657 mg. p.c. A varying proportion of reducing substances was made up of pentoses which ranged from 102 to 495 mg. p.c.

The results obtained from a series of determinations on skin flakes and head hair of the same individuals are summarized in Tables 2 and 3. In most instances total and amino acid nitrogen and pentoses were 6 to 12 times higher in skin flakes than in the corresponding hair. The increase in amino acid nitrogen after hydrolysis, though insignificant in hair, was marked in skin flakes.

TABLE 1.

Non-keratins of human skin flakes.

	Total N mg. p.c.	Urea N mg. p.c.	NH_3N mg. p.c.	Amino Acid N		Uric Acid mg. p.c.	Reducing Substances**		Pentoses mg. p.c.
				mg. p.c.	H* mg. p.c.		mg. p.c.	H* mg. p.c.	
(a)									
Average	1259 (3)	31	38	133 (2)	292 (2)	17 (3)	371 (2)	651 (2)	140 (2)
Range	1227-1316			119-147	268-316	11-23	362-380	450-852	102-177
(b)									
Average	1484 (17)	33 (3)	39 (3)	164 (7)	294 (7)	26 (14)	567 (7)	821 (7)	280 (11)
Range	1139-1831	28-42	31-45	141-202	200-379	10-56	400-728	513-1155	111-448
(c)									
Average	2253 (5)	155 (3)	102 (3)	348 (3)	494 (4)	39 (3)	808 (2)	937 (4)	389 (2)
Range	1844-2897	30-383	83-128	246-473	355-720	33-72	669-996	284-1657	283-495

(a) = Figures from normals.
(b) = Figures from hospital patients without renal involvement.
(c) = Figures from hospital patients with renal involvement and urea retention.
H* = After hydrolysis.
** = Expressed as glucose.
( ) = Numbers in brackets refer to number of specimens examined.

TABLE 2.

Average and range of non-keratins of skin flakes and hair from the same individuals.
(Cases from groups (a) and (b), Table 1.)

	Total (6) N mg. p.c.	Urea (3) N mg. p.c.	NH_3N(3) mg. p.c.	Amino Acid N (3) mg. p.c.	Amino Acid N (3) H* mg. p.c.	Uric Acid (6) mg. p.c.	Pentoses (6) mg. p.c.
Flakes							
Average	1310	33	38	151	226	23	326
Range	1139-1467	28-42	31-45	141-162	200-257	10-43	177-448
Hair							
Average	160	7	20	13	15	10	27
Range	122-245	5-9	13-30	10-23	10-25	8-13	17-35

H* = After hydrolysis.
( ) Numbers in brackets refer to number of specimens of flakes and hair examined.

TABLE 3.

Non-keratins of skin flakes and hair from an individual with moderate chronic nephritis.
(Case from group (c), Table 1, blood urea 58 mg. p.c.)

	Total N mg. p.c.	Urea N mg. p.c.	NH_3 N mg. p.c.	Amino Acid N mg. p.c.	Amino Acid N H* mg. p.c.	Uric Acid mg. p.c.	Pentoses mg. p.c.
Flakes	1844	51	96	326	461	34	495
Hair	357	40	133	74	74	15	32

H* = After hydrolysis.

DISCUSSION.

Mops of scalp hair, however well groomed, always yielded skin flakes when shaken up in ether and the production of skin flakes by the scalp is basically a normal process similar to that which occurs elsewhere on the body surface. The formation of dandruff in its simple form is frequently assumed to be an intensified process of skin flake production brought about by some micro-organism such as the *Pityrosporum ovale* of Mellassez. This so-called pityriasis simplex may continue without change into old age or may be transformed by further infection to pityriasis steatoides, and finally seborrhea oleosa. At present we are only concerned with simple dandruff without considering the causes of its qualitative and quantitative variations such as infection, endocrine factors, etc.

The qualitative composition of human skin flakes (dandruff) resembled that of other mammals and birds (Bolliger and Gross, 1954) and apparent differences encountered between man and animal may be caused by the comparatively small quantities of human skin flakes available for investigation. For example, the absence of histidine in the polypeptide obtained from dandruff as compared with its presence in the corresponding polypeptide from pigeons (Gross, 1955) may thus be explained. Histidine, however, could be demonstrated among the free amino acids of dandruff. The detection of cystine in the aqueous extract of skin flakes as a free amino acid as well as in the polypeptide molecule is to be emphasized in view of earlier statements of its absence in fur and hair extracts, skin wipings, etc. (Rothman, 1954; Bolliger and Gross, 1954a; Gross, 1955).

Comparison of human skin flakes and hair.

As in other mammals (Bolliger and Gross, 1954), the total non-keratin nitrogen was many times higher in human skin flakes than in the corresponding hair fibres (Table 2). To some extent this may be due to the lessened leaching out of skin flake non-keratins by the grooming process, because every specimen of dandruff collected represents mainly flakes accumulated since the last grooming. The persistently high soluble nitrogen values, however, make it quite clear that the skin flakes have a much higher non-keratin content than the formed keratinous appendages such as the hairs, which are situated in closest proximity. This phenomenon may be connected with the fact that the hair root possesses a vascular system whilst the keratinous skin possesses no such connection with the circulation.

In contrast to hair, free amino acid nitrogen was the largest fraction of the total non-protein nitrogen content of human skin flakes. On hydrolysis of the deproteinized extract the amino acid nitrogen content increased further and sometimes the free amino acid values became more than double after hydrolysis. This would suggest the presence of one or more polypeptides or other compounds containing amino acids in the aqueous extract not precipitated by tungstic acid. It is not known whether this is the same polypeptide which was precipitated by trichloracetic acid and whose qualitative amino acid composition has been examined by paper chromatography. On treatment with tungstic acid no precipitate was obtained from the aqueous extract of human hair and even very concentrated extracts only yielded traces of polypeptide-like substances with trichloracetic acid, confirming the analytical findings.

In the extracts of skin flakes are contained: (1) large amounts of free amino acids; (2) combined amino acids, not precipitated by sodium tungstate, and (3) polypeptides precipitated by tungstate. Cystine was shown to be present in (1) and (3). This would support the hypothesis that substances (1) and (3) are related to keratin formation. (3) might be a soluble form of keratin and (1) and (2) metabolic products of this protein.

Quantitative amino acid determinations by the method of Folin have been investigated by several authors (Frame, Russell and Wilhelmi, 1943), and with some amino acids errors up to 20 p.c. have to be expected. However, the method was found satisfactory for determining the overall amounts of amino acids in the deproteinized extracts.

A few determinations of urea and ammonia performed on the hair and skin flakes of the same individuals indicated that here the differences between skin flakes and hair were small. Uric acid, however, was higher in skin flakes than in hair. It is noteworthy that the uric acid content of hair and skin flakes of man is well below that found in other mammals in spite of the fact that man has a considerably higher uric acid content in the blood. The difference in the reducing substances was strikingly reflected by the pentose content which, on an average, was more than ten times higher in flakes than in hair. It is noticeable that in normals as well as in a variety of diseases fundamental quantitative differences between hair and skin flakes were always maintained (Tables 2 and 3).

In the present series of analyses it is difficult to distinguish sharply between normal and pathological values, but a definite trend towards nitrogen retention can be discerned in cases with renal insufficiency.

SUMMARY.

Cast off *stratum corneum* of the human scalp, referred to as skin flakes or simple dandruff, was collected from normals, hospital patients and cadavers and their water-extractable constituents (non-keratins) examined. The presence of 14 amino acids, free as well as combined as polypeptides, sugars, polysaccharides and purines was demonstrated.

Quantitative investigations included total nitrogen, urea, ammonia, uric acid, amino acids and reducing substances before and after hydrolysis and pentoses.

The composition of the non-keratins of human skin flakes resembles that of other mammalian cast off *stratum corneum* with the exception of a lower uric acid content. Compared with human hair, the total nitrogen content of dandruff is many times higher, which is partly accounted for by the presence of polypeptides and an increase in the free amino acid nitrogen.

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LYSOGENICITY AND LYSIS PATTERNS IN THE SALMONELLAS

6. THE OCCURRENCE OF *S. BOVIS-MORBIFICANS* OF PHAGE GROUP 6

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By lysogenicity and lysis tests Atkinson and Klauss (1955) defined three provisional phage groups (numbered 4, 5, and 6) of *S. bovis-morbificans*. They noted the prevalence of strains of phage group 6 among those obtained from Brisbane during the epidemic of gastro-enteritis of 1947, described by Mackerras and Mackerras (1949). Further to investigate this apparent connection of group 6 strains with the Brisbane epidemic, 63 more Brisbane strains of the epidemic period were tested, as well as 29 more strains from other places or from Brisbane after the epidemic period. The results of this investigation are recorded here and support the suggested connection of group 6 strains with the Brisbane epidemic.

MATERIALS AND METHODS.

The 92 strains of *S. bovis-morbificans* investigated here consisted of 63 strains selected at random from numerous cultures received from Brisbane during the epidemic period from September, 1947, to March, 1948, 19 strains received from Sydney and Adelaide mainly in 1953, and 10 strains received from Brisbane after the epidemic period and mainly in 1953. As the 80 strains of *S. bovis-morbificans* previously investigated (Atkinson *et al.*, 1952; Atkinson and Klauss, 1955) were numbered from 1–80, the new batch of 92 strains was allotted numbers from 81 to 180. The strain numbers together with origins and dates of isolation are given in Table 1, which also includes the results of lysis and lysogenicity tests.

The lysis and lysogenicity tests were made by the methods described by Atkinson and Klauss (1955). The phage preparation BM14/18 used in lysis tests consisted of a purified preparation from an isolated plaque from a heated supernatant of a broth extract of the mixed growth of *S. bovis-morbificans* strains 14 and 18 on agar. This phage was heat resistant and was therefore freed from living bacteria by heating at 56° C. for 30 minutes. In the lysogenicity tests the 92 strains were tested against the three type strains 18, 14 and 8 of groups 4, 5 and 6 respectively, described by Atkinson and Klauss (1955). In Table 1 the results of these lysogenicity tests are recorded as + if the extract of the mixed growth of test and indicator strain lysed the indicator strain, or − if the extract failed to lyse the indicator strain.

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The 92 strains were also tested for lysogenicity among themselves in groups of about eight in all combinations of pairs. Similar strains could be detected here as they showed no lysogenic action on each other.

RESULTS.

The results obtained with the 92 strains in lysis tests with phage BM14/18 and lysogenicity tests with strains 18 (group 4), 14 (group 5) and 8 (group 6) are given in Table 1.

Group 6 strains were distinguished by their failure to lyse with phage BM14/18, their mutual lysogenicity with strains 18 (group 4) and 14 (group 5), and their failure to show any lysogenicity with strain 8 (group 6). From the results in Table 1, in which the lysogenicity patterns of the 92 strains on strains 18 (group 4), 14 (group 5) and 8 (group 6) are recorded, strains of group 6 could be distinguished. However, the lysogenicity patterns of strains 18 (group 4), 14 (group 5) and 8 (group 6) on the 92 strains were also determined and supported the groupings given in Table 1. Thus all strains placed in group 6 in Table 1 were lysed by strains 18 and 14 with the tiny clear plaques they typically produced on strains of group 6, but strain 8 produced no lysis.

Furthermore, in the lysogenicity tests on batches of test strains in all combinations of pairs, any group 6 strains in a batch were mutually non-lysogenic. The placing of a strain in group 6, therefore, though depending primarily on the results in Table 1, always had the additional support of the lysogenicity patterns with strains 18, 14 and 8 and with other test strains placed in group 6.

Of the 63 strains from Brisbane during the epidemic period, strain 88 was not tested on account of phage-eaten growth, strains 86, 91, 94, 102, 103, 110, 112, 126, 128, 132 and 149 were ungrouped, and the remaining 51 strains gave the reactions of group 6. Of the 19 strains from Sydney and Adelaide, six strains (152, 154, 155, 156, 165 and 169) from Adelaide gave the reactions of group 6, one strain (153) from Adelaide gave the reactions of group 4 and the remaining 12 strains were ungrouped. Of the 10 strains from Brisbane, long after the epidemic period, one strain (180) gave the reactions of group 4, one strain (178) gave the reactions of group 5, and the remaining eight strains were ungrouped. These figures indicated the predominance of group 6 strains in Brisbane during the epidemic period, but not at a later date, and also showed that group 6 strains occurred in Adelaide.

DISCUSSION.

From the results presented here of the investigation of 92 strains of *S. bovis-morbificans* from Brisbane, Sydney and Adelaide, group 6 strains were undoubtedly more common in Brisbane during the epidemic period of 1947.

TABLE 1.

Lysis and lysogenicity tests with 92 *strains of* S. bovis-morbificans.

Strain no.	Source	Date of isolation	Lysis with phage BM14/18	Lysogenicity on			Grouping
				Strain 18 (group 4)	Strain 14 (group 5)	Strain 8 (group 6)	
63 strains from Brisbane during the epidemic period of 1947.							
81	cockroach	11/47	−	+	+	−	group 6
82	cockroach	11/47	−	+	+	−	group 6
83	mouse colon	9/47	−	+	+	−	group 6
84	mouse liver	9/47	−	+	+	−	group 6
85	mouse colon	9/47	−	+	+	−	group 6
86	rat spleen	9/47	−	+	+	+	ungrouped
88	scrubbing brush	11/47	0	0	0	0	not tested phage infected
89	patient (mastoid)	11/47	−	+	+	−	group 6
90	patient (faeces)	9/47	−	+	+	−	group 6
91	patient ,,	11/47	−	+	+	+	ungrouped
92	patient ,,	11/47	−	+	+	−	group 6
93	patient ,,	11/47	−	+	+	−	group 6
94	patient ,,	10/47	−	+	+	+	ungrouped
95	patient ,,	8/47	−	+	+	−	group 6
97	patient (small intestine)	9/47	−	+	+	−	group 6
98	patient (faeces)	11/47	−	+	+	−	group 6
99	patient ,,	11/47	−	+	+	−	group 6
100	patient ,,	9/47	−	+	+	−	group 6
101	patient ,,	11/47	−	+	+	−	group 6
102	patient ,,	12/47	−	+	+	+	ungrouped
103	patient ,,	10/47	−	+	+	+	ungrouped
104	patient ,,	11/47	−	+	+	−	group 6
106	patient ,,	11/47	−	+	+	−	group 6
107	patient ,,	10/47	−	+	+	−	group 6
108	patient ,,	12/47	−	+	+	−	group 6
109	patient (colon)	9/47	−	+	+	−	group 6
110	patient (faeces)	11/47	+	+	+	+	ungrouped
112	patient ,,	12/47	−	−	−	+	ungrouped
113	patient ,,	11/47	−	+	+	−	group 6
114	patient (colon)	10/47	−	+	+	−	group 6
115	patient (faeces)	11/47	−	+	+	−	group 6
116	patient ,,	9/47	−	+	+	−	group 6
117	patient (liver)	9/47	−	+	+	−	group 6
119	patient (middle ear)	9/47	−	+	+	−	group 6
120	patient (colon)	12/47	−	+	+	−	group 6
121	patient (faeces)	11/47	−	+	+	−	group 6
123	patient ,,	10/47	−	+	+	−	group 6
124	patient ,,	11/47	−	+	+	−	group 6
125	patient ,,	11/47	−	+	+	−	group 6
126	patient ,,	8/47	+	+	+	+	ungrouped
127	patient ,,	9/47	−	+	+	−	group 6
128	patient ,,	8/47	−	+	+	+	ungrouped
129	patient ,,	9/47	−	+	+	−	group 6
130	patient ,,	11/47	−	+	+	−	group 6
132	patient ,,	11/47	−	+	+	+	ungrouped
133	patient ,,	12/47	−	+	+	−	group 6
134	patient ,,	10/47	−	+	+	−	group 6
135	patient ,,	11/47	−	+	+	−	group 6

TABLE 1—*continued.*

Strain no.	Source	Date of isolation	Lysis with phage BM14/18	Lysogenicity on			Grouping
				Strain 18 (group 4)	Strain 14 (group 5)	Strain 8 (group 6)	
136	patient (spleen)	9/47	−	+	+	−	group 6
137	patient (faeces)	11/47	−	+	+	−	group 6
138	patient ,,	10/47	−	+	+	−	group 6
139	patient ,,	11/47	−	+	+	−	group 6
140	patient ,,	12/47	−	+	+	−	group 6
141	patient (spleen)	9/47	−	+	+	−	group 6
142	patient (faeces)	12/47	−	+	+	−	group 6
143	patient (ileum)	9/47	−	+	+	−	group 6
144	patient (liver)	12/47	−	+	+	−	group 6
145	patient (faeces)	11/47	−	+	+	−	group 6
146	patient ,,	10/47	−	+	+	−	group 6
147	patient (spleen)	9/47	−	+	+	−	group 6
148	patient (faeces)	10/47	−	+	+	−	group 6
149	patient ,,	8/47	−	+	+	+	ungrouped
150	patient ,,	11/47	−	+	+	−	group 6
19 strains from Sydney and Adelaide							
151	patient (faeces) Sydney	4/53	−	+	+	+	ungrouped
152	patient (faeces) Adelaide	2/55	−	+	+	−	group 6
153	patient (faeces) Adelaide	2/53	+	−	−	+	group 4
154	patient (faeces) Adelaide	2/53	−	+	+	−	group 6
155	patient (faeces) Adelaide	3/53	−	+	+	−	group 6
156	patient (faeces) Adelaide	3/55	−	+	+	−	group 6
157	patient (faeces) Sydney	11/53	−	+	+	+	ungrouped
159	patient (faeces) Sydney	4/55	−	+	+	+	ungrouped
160	patient (faeces) Sydney	5/53	−	+	+	+	ungrouped
161	patient (faeces) Sydney	5/53	−	+	+	+	ungrouped
162	patient (faeces) Sydney	5/53	−	+	+	+	ungrouped
163	patient (faeces) Sydney	5/53	−	+	+	+	ungrouped
164	patient (faeces) Adelaide	11/53	−	+	+	+	ungrouped
165	patient (faeces) Adelaide	5/55	−	+	+	−	group 6
166	patient (faeces) Sydney	5/53	−	+	+	+	ungrouped
167	patient (faeces) Sydney	5/53	−	+	+	+	ungrouped
168	patient (faeces) Adelaide	11/53	−	+	+	+	ungrouped
169	guinea pig Adelaide	4/55	−	+	+	−	group 6
170	patient (faeces) Sydney	5/52	−	+	+	+	ungrouped
10 strains from Brisbane after the epidemic period of 1947-1948.							
171	patient (faeces)	1/53	+	+	+	+	ungrouped
172	patient (faeces)	1/53	+	+	+	+	ungrouped
173	patient (faeces)	3/53	+	+	+	+	ungrouped
174	rat	3/53	+	+	+	+	ungrouped
175	rat	3/53	+	+	+	+	ungrouped
176	patient (faeces)	9/53	+	+	+	+	ungrouped
177	patient (faeces)	9/53	+	+	+	+	ungrouped
178	patient (faeces)	10/53	−	+	−	+	group 5
179	pig intestine	5/55	−	+	+	+	ungrouped
180	mouse	2/52	+	−	−	+	group 4

+ = confluent or semiconfluent lysis or many plaques.
− = no lysis detected.
0 = not tested.

(51 out of 62 strains tested) than at a later date (none out of 10 tested). The group 6 strains seem therefore to have been mainly responsible for the epidemic. Besides occurring in the majority of patients investigated here, they were also found in cockroaches and mice (strains 81, 82, 83, 84) caught in the hospital where many patients were treated. Strain 86, from a rat unconnected with the hospital, was not placed in group 6. In Adelaide group 6 strains sporadically appeared. Atkinson and Klauss (1955) recorded two group 6 strains from Adelaide and, in the present paper, six out of nine strains from Adelaide were placed in group 6. No members of group 6 were found among 10 strains from Sydney. Apparently group 6 strains are endemic in Adelaide but so far have not been responsible for any major epidemic.

The methods of testing used here allowed the detection of group 4 and group 5 strains also. Two group 4 strains (153 and 180) and one group 5 strain (178) were found. These groups were rare and only two group 4 strains (18 and 54) and two group 5 strains (14 and 44) had previously been found (Atkinson *et al.*, 1952; Atkinson and Klauss, 1955).

SUMMARY.

Using one phage preparation and three indicator strains, the lysogenicity and lysis patterns of 92 strains of *S. bovis-morbificans* were determined. From these patterns three phage groups (4, 5 and 6) could be distinguished. Of 63 strains from Brisbane during the epidemic of 1947, 51 were placed in group 6. No group 6 strains were found, however, among 10 strains from Brisbane at a later date. Group 6 strains were therefore considered to have been mainly responsible for the Brisbane epidemic. No group 6 strains were found among ten from Sydney, but six out of nine strains from Adelaide were placed in group 6. Two group 4 strains and one group 5 strain were also found. These two groups were rare.

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LYSOGENICITY AND LYSIS PATTERNS IN THE SALMONELLAS

7. A BACTERIOPHAGE GROUPING SCHEME FOR *S. BOVIS-MORBIFICANS*

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In a preliminary investigation, Atkinson *et al.* (1952) found that 60 strains of *S. bovis-morbificans* were all lysogenic when tested among themselves. Four groups (1, 2, 3 and 4) were tentatively recognised and 22 strains were grouped. Later Atkinson and Klauss (1955) described two more provisional groups (5 and 6) to which 16 of the previously ungrouped strains were allocated. A bacteriophage grouping scheme has now been developed for *S. bovis-morbificans* and is presented here. This scheme resembled that already described for *S. adelaide* (Atkinson, 1955) and depended upon lysis and lysogenicity patterns with type phages and selected indicator strains. The patterns were more complex than those of *S. adelaide* and many strains of *S. bovis-morbificans* could not be grouped. Obviously the scheme must eventually be modified to accommodate these ungrouped strains when their characteristics become clearer.

Methods.

The methods were those used for the *S. adelaide* phage grouping scheme (Atkinson, 1955) except that phage preparations were diluted sufficiently to remove the contaminating phage of the lysogenic propagating strain. This phage was present only in the small amounts given off by the propagating strain and was easily diluted out. The lowest dilution in which the contaminating phage was no longer detectable was called the test dilution and was determined by lysis tests with serial dilutions of the phage preparation on an indicator strain distinguishing the contaminating phage from the main phage. Thus for phage preparations propagated on *S. bovis-morbificans* strain 18 the test dilution was determined on strain 8, and for phage preparations propagated on strain 8 or 14 the test dilution was determined on strain 18.

THE PHAGE GROUPING SCHEME.

The scheme contained five groups (2, 3, 4, 5 and 6). The tentative group 1 (Atkinson *et al.*, 1952) was not included as it appeared heterogeneous. The groups were defined by both lysis and lysogenicity tests. Strains to be grouped

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were tested for lysis with five type phages and for lysogenicity with five indicator strains. The five type phages were BM17/18 (type 2), BM45/18 (type 3), BM14/8 (type 4), BM14/18 (type 5) and BM8/14 (type 6). These phages will be described in a separate paper. They were all derived from strains of *S. bovis-morbificans*. The five indicator strains represented the five phage groups for which they had been selected as the type strains. They were *S. bovis-morbificans* strains 17 (group 2), 45 (group 3), 18 (group 4), 14 (group 5) and 8 (group 6). Strains 17, 45, 18 and 14 were briefly mentioned by Atkinson *et al.* (1952), and strains 18, 14 and 8 were more extensively described by Atkinson and Klauss (1955). These strains will be further defined in a separate paper.

Table 1 presents the reactions of the type strains of the five phage groups with the phages and indicator strains of the grouping scheme.

In Table 1 may be seen the typical lysis and lysogenicity patterns of the five groups.

Group 2 (strain 17) was distinguished by (a) its lysis with types 3, 5 and 6 phages and its resistance to types 2 and 4 phages, (b) its lysogenic action on the type strains of all the other groups 3, 4, 5 and 6, (c) the lysogenic action on it of the type strains of groups 3, 5 and 6.

Group 3 (strain 45) was distinguished by (a) its lysis with types 2, 5 and 6 phages and its resistance to types 3 and 4 phages, (b) its lysogenic action on the type strains of all the other groups 2, 4, 5 and 6, (c) the lysogenic action on it of the type strains of groups 2, 5 and 6.

Group 4 (strain 18) was distinguished by (a) its lysis with all the type phages except type 4, (b) its lysogenic action on group 6 only, (c) the lysogenic action on it of the type strains of all the other groups 2, 3, 5 and 6.

Group 5 (strain 14) was distinguished by (a) its lysis with types 2, 3 and 6 phages and its resistance to types 4 and 5 phages, (b) its lysogenic action on the type strains of all the other groups 2, 3, 4 and 6, (c) the lysogenic action on it of the type strains of groups 2, 3 and 6.

Group 6 was distinguished by (a) its lysis with types 2 and 4 phages and its resistance to types 3, 5 and 6 phages, (b) its lysogenic action on the type strains of all the other groups 2, 3, 4 and 5, (c) the lysogenic action on it of the type strains of all the other groups 2, 3, 4 and 5.

The type of phage carried by each group is also recorded in Table 1. Groups 2 and 3 each carried a phage (types 2 and 3 respectively) which was distinctive for the group. Group 4 carried a phage (type 4) which was also found in group 5. Group 5 differed from group 4, however, in carrying a second phage (type 5). Group 6 resembled group 5 in carrying type 5 phage but was distinguished from group 5 by carriage of a second phage (type 6). The lysis and lysogenicity patterns between these three groups, 4, 5 and 6, were directly related to phage carriage.

TABLE 1.

Reactions of five type strains of S. bovis-morbificans *in the proposed phage grouping scheme.*

Proposed phage group no.	Type strain no.	Lysis with phage					Lysogenicity with strain					Type of phage carried
		BM17/18 (type 2)	BM45/18 (type 3)	BM14/8 (type 4)	BM14/18 (type 5)	BM8/14 (type 6)	17 (group 2)	45 (group 3)	18 (group 4)	14 (group 5)	8 (group 6)	
2	17	−	+	−	+	+	−	±	+	+	+	type 2
3	45	±	−	−	+	±	+	−	+	+	+	type 3
4	18	+	+	−	+	+	−	−	−	−	+	type 4
5	14	+	+	−	−	+	+	+	+	−	+	type 4 and type 5
6	8	+	−	+	−	−	+	+	+	+	−	type 5 and type 6

+ = confluent or semi-confluent lysis or many plaques.
± = a few plaques.
− = no lysis.

DISCUSSION.

In the phage grouping scheme proposed for *S. adelaide* (Atkinson, 1955) the subdivisions were called phage groups rather than phage types as they were determined by lysogenicity as well as lysis patterns. For the same reason, the subdivisions of the proposed phage grouping scheme for *S. bovis-morbificans* were also called phage groups.

Of the five phage groups suggested for *S. bovis-morbificans*, groups 2, 3, 5 and 6 were mutually lysogenic. Group 4 showed mutual lysis only with group 6. It was readily lysed by the other groups 2, 3 and 5 but failed to lyse them. Group 4 therefore provided good propagating and indicator strains for the phages of the other groups. For the phage of group 4, however, group 6 provided the only propagating strains.

The lysis and lysogenicity patterns of groups 4, 5 and 6 directly reflected the carriage phages, but in groups 2 and 3 resistance to type 4 phage has not yet been related to phage carriage.

The proposed phage grouping scheme was applied to 170 strains of *S. bovis-morbificans* of which 112 have so far been grouped. These results will be published in a separate paper. The complex lysogenicity patterns of the ungrouped strains indicated that several new phage groups awaited recognition and inclusion in the proposed grouping scheme.

SUMMARY.

On the basis of lysogenicity and lysis tests, a bacteriophage grouping scheme for *S. bovis-morbificans* was devised. The lysis patterns were determined with five *S. bovis-morbificans* type phages and the lysogenicity patterns with five indicator strains of *S. bovis-morbificans*. The scheme contained five groups with distinctive patterns.

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THE EFFECT OF PITUITARY POSTERIOR LOBE EXTRACTS ON THE BLOOD PRESSURE OF THE PIGEON

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Pressor and oxytocic effects of posterior lobe pituitary extracts are assayed biologically by comparison with international standard powder. The assay of the former is performed by comparison of blood pressure increments in anaesthetized or spinal mammals and of the latter in terms of the contraction of the mammalian uterus *in vitro* or by its depressor property in anaesthetized fowls. Measurement of oxytocic properties, using the anaesthetized fowl, has been gaining favour because of its simplicity and reliability.

The present investigation was undertaken to see whether the pigeon could replace the fowl; this was both because fowls were unobtainable in England at that time and also because we hoped that the pigeon might be more sensitive.

The primary object of the investigation failed; among other things, but of over-riding importance when considering a routine procedure, the manipulations proved to be too tedious. On the other hand, some responses were recorded which raised questions about responses in the fowl. Consequently fowl responses were re-investigated, and now that these have been recorded (Strahan and Waring, 1954) it is appropriate to record briefly responses of the pigeon and how these differ from those of the fowl.

METHODS.

Pigeons of about 250 gms. were anaesthetized with ether and a rectal temperature of 39–42° C. maintained. The trachea was cannulated in orthodox fashion and the brachial vein cannulated with a 22 S.W.G. needle connected at its base to a short closed section of pressure tubing into which injections were made. 70 mg./kg. body weight of soluble pentobarbitone were slowly injected intravenously over a period of about an hour; this dose is sufficient to stop respiration, and when this happened artificial respiration at a pump speed of 40 strokes per minute was applied. The preparation was heparinized, the femoral artery cannulated and connected to a mercury manometer by a column of citrate. All doses injected were approximately 0·2 c.c. washed in with the same volume of saline.

RESULTS.

Response to pitocin.

The response to pitocin was invariably a sharp fall in pressure followed by a slower rise to the original pressure and a secondary rise. The secondary

rise is of a size that could be attributed to the known pressor contamination in the pitocin. We found the best discrimination (10 p.c.) to be in the neighbourhood of a dose of 0·07 international units, which gave an excursion of about 35 mm. Hg.

Response to pitressin.

Pitressin evokes a rise in blood pressure. The response is graded and discrimination between doses 10 p.c. different is possible with doses of the order of 0·003 i.u., which give an excursion of about 35 mm. The response to much larger doses is also a rise, but responses are erratic.

Responses to mixtures of pitressin and pitocin.

Mixtures of pitressin and pitocin evoke a fall followed by a rise in pressure. The greater the relative concentration of pitocin, the greater is the fall and the less the rise.

Response to unfractionated whole extract of posterior lobe pituitary.

Unlike mixtures of pitressin and pitocin, initial injections of unfractionated extract evoke a sharp fall with no following rise. The response to succeeding doses of the same extract is complicated and has not been analysed.

Response to pitressin after intravenous injection of magnesium chloride.

Intravenous injection of magnesium chloride (10·5 to 1·0 c.c. of 0·048 p.c. solution) alters the pressor action of pitressin. With doses of the latter less than 0·01 i.u. the pressor effect is merely reduced; with doses more than 0·02 i.u. there is a pure fall to a dose immediately after the magnesium injection, but 15 minutes later the response to injection is the usual rise.

Response to histamine and adrenaline.

Histamine evoked the expected fall and the preparation is sufficiently sensitive to it for the usual histamine contamination of commercial powders (2 p.c. by weight) to reduce the pitressin rise measurably.

The preparation is very sensitive to adrenaline, responding by a rise of about 40 mm. to a dose of 0·004 mg. adrenaline hydrochloride. Discrimination is shown between doses of this order, differing by 10 p.c.

DISCUSSION.

The object of this investigation was to test the value of the pigeon for oxytocic assay. A good fowl preparation will respond measurably to 0·01 i.u. of pitocin, and in routine work one can expect to use doses of about 0·1 i.u. for assays. The results reported above show that, on score of sensitivity, the pigeon preparation offers no advantage. The pigeon preparation is so tedious to set up that it cannot be recommended for routine assays.

The chief matter of interest that emerged, and which caused us to make a re-investigation of fowl responses (Strahan and Waring, 1954), is the response to pitressin. A fowl with low blood pressure responds to pitressin with a rise; with high blood pressure the response is a fall. With the pigeon we always recorded a rise, and in some of the preparations the base pressures were as high as 12 cm. Hg. The effect of augmenting the plasma magnesium prior to pitressin injection was substantially similar to what occurs in the fowl, i.e., according to dosage the pressor response is reduced or converted to a fall.

A cognate matter of interest is the response to whole extract as compared with the response to mixtures of pitressin and pitocin. Unfractionated extract of posterior lobe pituitary has both pressor and oxytocic properties. Pitressin and pitocin are fractions derived from whole gland with, respectively, substantially pressor only and oxytocic only properties. Recently (du Vigneaud, 1953) pure substances with properties approximating to pitressin and pitocin have been obtained. There is, however, some evidence that the pressor and oxytocic activities are loosely bound together in a carefully prepared extract not subjected to procedures likely to cause hydrolysis (Waring and Landgrebe, 1950). If this is true we might expect different responses from whole extract and a mixture of pitressin and pitocin, under circumstances where a preparation is reacting in opposite fashion to pitressin and pitocin. Observations are available in three such cases.

(a) *Fowl.* With low blood pressure fowls exhibit a rise to pitressin and a fall to pitocin and a rise and fall to mixtures (Strahan and Waring, 1954). Hogben and Schlapp (1924) reproduce traces from five fowls injected with whole extract with a base pressure at which a rise to pitressin would result; all five exhibited depression and only two had rises following.

(b) *Platypus* (Feakes *et al.*, 1950) exhibits a rise to pitressin, a fall to pitocin, and a rise and fall to mixtures. Injection of whole extract causes a fall followed by only a small rise.

(c) *Pigeon.* As recorded above, response to whole extract is a fall with no following rise, but to mixtures there is a fall to the pitocin and rise to the pitressin. These observations support the other evidence put forward by Waring and Landgrebe (1950), suggesting that the pressor and oxytocin components of powder carefully prepared from the whole gland are bound together so that a simple extract does not necessarily behave in the same way as a mixture of pitressin and pitocin.

SUMMARY.

The responses of the anaesthetized pigeon to pituitary extract were investigated with a view to using them for assay purpose. The preparation is not recommended.

The response to pitressin is a rise of blood pressure, and to pitocin a fall.

Prior injection of magnesium chloride can convert a pitressin rise into a pure fall.

The responses of the pigeon to pituitary are compared with those of the fowl.

The preparation is sensitive both to histamine and adrenaline.

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